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Improving Staging Accuracy in Melanoma

Niebling, Maarten Gert-Jan

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Improving Staging Accuracy in Melanoma

M.G. Niebling

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Paranimfen:	Arne Bleeker
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Chapter 1

General introduction and outline of thesis

Incidence of cutaneous melanoma

The incidence of cutaneous melanoma has increased dramatically in Caucasian populations in all parts of the world; it is one of the tumors with the most rapidly increasing incidence among all malignancies.[1-5] Australia has the highest incidence of melanoma in the world with an incidence rate of 48.8 per 100 000 in 2008.[6] In the Netherlands incidence of cutaneous melanoma is lower (25 per 100 000 in 2008), but it is rising rapidly since incidence rates have almost doubled in the last decade (17.3 per 100 000 in 2002 and 30.4 per 100 000 in 2011).[7] The rising incidence is partly due to increased awareness in the general population, melanoma is now more often diagnosed at an earlier stage, as the median Breslow thickness at first clinical presentation declined over time and the majority of patients are diagnosed with stage I or II melanoma.[8] However, 1 out of 6 melanoma patients will succumb within 10 years due to progression of their melanoma.[7]

The staging of primary cutaneous melamoma (stage I and II)

The 7th edition of the TNM staging guidelines for melanoma was developed in 2009 by the American Joint Committee on Cancer (AJCC),[3] Tables 1 and 2 show the TNM classification and anatomic stage groupings for melanoma. The T-classification can be determined by pathologic features of the primary cutaneous melanoma: Breslow thickness, ulceration, and tumor mitotic rate.[9,10] Clark level of invasion is no longer recommended as a primary staging criterion for T1 melanomas, since it is not an independent prognostic factor when tumor mitotic rate is included in the pathological analysis. However, it can still be used as a criterion for T1 melanomas if tumor mitotic rate and ulceration cannot be determined.[3] Microsatellites of the primary cutaneous melanoma is also an important pathologic feature of the primary melanoma and is used for N2c staging in the TNM classification of melanoma, because the presence of microsatellites is associated with an increased frequency of lymph node metastasis.[9,11]

Treatment of primary cutaneous melanoma

The most important factor for successful management of melanoma is early diagnosis, allowing treatment to be undertaken at a stage when cure is still achievable.[12] Management of primary cutaneous melanoma is largely dependent on diagnosis and T-stage of melanoma. Based on randomized controlled trials,[13-23] both Dutch[24] and other national guidelines[25] recommend a surgical excision with a margin of 0.5cm margin for in-situ melanoma, a margin of 1cm for T1-T2 invasive melanomas, and a margin of 2 cm for T3-T4 invasive melanomas.

The staging and treatment of melanoma patients with nodal metastases (stage III)

The N-classification is primarily based on melanoma metastases in the regional lymph nodes (Figure 1). The lymphatic route is a principal way of spread of melanoma cells from their original focus, the melanoma cells progressing via the lymphatic vessels are stopped in the first node on the way: the sentinel lymph node (SLN). Lymph node metastases can be detected clinically or with a sentinel lymph

Table 1. TNM staging categories for cutaneous melanoma

Tumor	**Breslow thickness**	**Ulceration / mitotic rate**
T1	≤1.0 mm	T1a: Without ulceration and mitosis <1/mm^2
		T1b: With ulceration and mitosis ≥1/mm^2
T2	1.01-2.0 mm	T2a: Without ulceration
		T2b: With ulceration
T3	2.01-4.0 mm	T3a: Without ulceration
		T3b: With ulceration
T4	>4.0 mm	T4a: Without ulceration
		T4b: With ulceration
Node	**No. of metastatic nodes**	**Nodal metastatic burden***
N0	0	
N1	1	N1a: Micrometastasis
		N1b: Macrometastasis
N2	2-3	N2a: Micrometastases
		N2b: Macrometastases
		N2c: In transit metastases/satellites without metastatic nodes
N3	4+ metastatic nodes, matted nodes, or in transit metastases/satellites with metastatic nodes	
Metastasis	Site	Serum LDH
M0	No distant metastasis	
M1a	Distant skin, subcutaneous, or nodal metastases	Normal LDH
M1b	Lung metastases	Normal LDH
M1c	All other visceral metastases	Normal LDH
	Any distant metastasis	Elevated LDH

* Micrometastases are diagnosed through sentinel lymph node biopsy. Macrometastases are defined as clinically detectable nodal metastases confirmed pathologically.

Table 2. Anatomic stage groupings for melanoma

	Clinical Staging*				Pathologic Staging*			
	T	N	M		**T**		**N**	**M**
IA	T1a	N0	M0	**IA**	T1a		N0	M0
IB	T1b	N0	M0	**IB**	T1b		N0	M0
	T2a	N0	M0		T2a		N0	M0
IIA	T2b	N0	M0	**IIA**	T2b		N0	M0
	T3a	N0	M0		T3a		N0	M0
IIB	T3b	N0	M0	**IIB**	T3b		N0	M0
	T4a	N0	M0		T4a		N0	M0
IIC	T4b	N0	M0	**IIC**	T4b		N0	M0
III	Any T	N1-3	M0	**IIIA**	T1-4a		N1a	M0
					T1-4a		N2a	M0
				IIIB	T1-4b		N1a	M0
					T1-4b		N2a	M0
					T1-4a		N1b	M0
					T1-4a		N2b	M0
					T1-4a		N2c	M0
				IIIC	T1-4b		N1b	M0
					T1-4b		N2b	M0
					T1-4b		N2c	M0
					Any T		N3	M0
IV	Any T		Any N	M1	IV	Any T	Any N	M1

* Clinical staging includes microstaging of the primary melanoma and clinical/radiologic evaluation for metastases. By convention, it should be used after complete excision of the primary melanoma with clinical assessment for regional and distant metastases. Pathologic staging includes microstaging of the primary melanoma and pathologic information about the regional lymph nodes after partial (ie, sentinel node biopsy) or complete lymphadenectomy. Pathologic stage 0 or stage IA patients are the exception; they do not require pathologic evaluation of their lymph nodes.

node biopsy (SLNB). SLNB is a widely accepted staging procedure and is performed in patients with a melanoma ≥1mm thick, or a melanoma <1mm thick with presence of ulceration and/or a tumor mitotic rate ≥1.[9,26] The interim analysis of the Multicenter Selective Lymphadenectomy Trial I (MSLT-I trial) already showed that the sentinel node status is a very important prognostic indicator in patients with clinically localized melanoma.[27] A survival benefit of SLNB is yet to be shown. The final data from the MSLT-I study will provide information on the definitive survival benefit of SLNB.[28]

According to the incubator hypothesis, the primary melanoma spreads primarily to sentinel nodes in the regional lymph basin, where the metastatic melanoma cells may survive and grow slowly or remain latent before, in some patients, spreading to distant sites.[29] If the regional metastatic disease could be removed prior to systemic spread, the patient would be cured. Melanoma patients with regional metastases can be treated by surgery with or without adjuvant systemic and/or radiation therapy.[30,31] Melanoma patients with a positive SLNB are surgically treated with either a Completion Lymph Node Dissection (CLND) or watchful waiting in the Multicenter Selective Lymphadenectomy Trial II (MSLT II trial).[32,33] Clinically detected lymph node metastases (LNM) are treated with a Therapeutic Lymph Node Dissection (TLND) in order to achieve locoregional control.[34] Adjuvant radiotherapy is recommended depending on the number of LNM, the size of the largest lymph node metastasis, and the presence of extranodal growth.[30] The 5-year survival ranges from 40-78% for stage III melanoma patients.[9,35] Patients with clinically detected lymph node metastases tend to have worse survival than patients with lymph node metastases detected with SLNB (5-year survival: 43% vs 67%).[35]

The staging and treatment of melanoma patients with distant metastases (stage IV)

Distant metastases of melanoma (stage IV) are grouped in the M-classification, categorized according to location and level of serum Lactate Dehydrogenase (LDH). Stage IV melanoma patients have the worst prognosis with 5-year survival rates ranging from 4.9-11%.[36-39] Prognostic factors for the survival of stage IV melanoma patients are the location and number of distant metastases, and serum level of biomarkers including S-100B and LDH.[9,40-42] Systemic medical treatment with an anti-CTLA4 antibody, Ipilimumab; BRAF inhibitors (including Vemurafinib or Dabrafenib); and MEK inhibitors (including Trametinib) have shown promising results in the treatment of stage IV melanoma patients.[43-45] When surgery is feasible for stage IV melanoma patients this should be the first choice of treatment with a curative

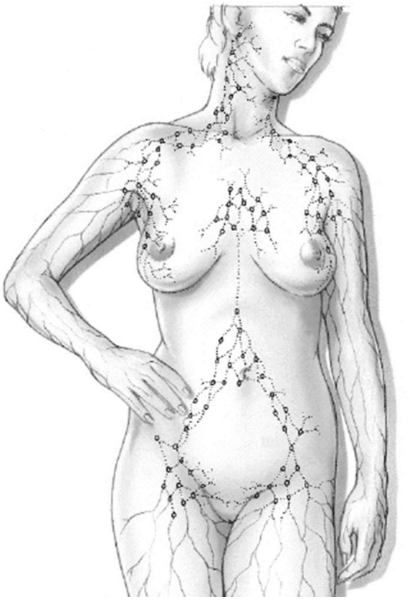

Figure 1. Draining lymph node fields (Source: Dutch Cancer Society)

intent, as 5-year survival rates have shown to be between 15-42%.[40,41,46-54] However, a recent Dutch study showed that only a few metastasized melanoma patients are candidates for surgical resection.[55]

Imaging techniques in melanoma

Imaging with ultrasound, X-ray, computed tomography (CT), magnetic resonance imaging (MRI), and especially nuclear imaging play an important role in staging of melanoma patients.

Nuclear imaging: PET/CT, lymphoscintigraphy, and SPECT/CT

Fluorodeoxyglucose (FDG) positron emission tomography (PET) combined with CT is a valuable nuclear imaging tool to screen for metastases since melanoma typically is very FDG avid and since a whole body scan covers the full, often erratic pattern of spread.[56] Meta-analyses performed to examine the utility of FDG-PET/CT, FDG-PET alone, and CT alone for the staging and surveillance of patients with melanoma based on 10,528 patients between 1990 and 2009 found FDG-PET/CT to be the most accurate modality for the detection of distant metastases.[57] However, uptake of FDG is also seen in inflammation and infection, but also by muscles and the central nervous system and therefore FDG-PET sensitivity is lower in detecting melanoma foci in lung, liver, and brain.[58] Images of FDG-PET and FDG-PET/CT of a melanoma patient with distant metastases are presented in *Chapter 8* of this thesis. In stage I, II, and microscopic stage III patients, FDG-PET/CT has no additional value.[58-60] However, FDG-PET/CT does have additional value in treatment planning for patients with palpable, proven lymph node metastases with no suspicion for lung metastases on X-ray.[59,61] FDG-PET/CT may also be of importance for stage IV melanoma patients to localize the distant metastases if surgical treatment is considered.[58,59] Molecular imaging techniques are being improved to overcome specificity issues with FDG, and to enhance signal-to-background ratios by improving targeting and/or capitalizing on lower background tracer uptake. With the use of new tracers like ^{18}F-ICF01006 the detection of both lymph node and distant metastases with PET/CT could improve.[62] Furthermore, numerous of other promising melanoma specific tracers are being investigated.[63-65]

Lymphoscintigraphy and SPECT/CT in SLNB

Lymphoscintigraphy is an imaging technique that has proven to be of use for sentinel lymph node mapping and is performed before SLNB.[58] It is used to identify the lymph drainage basin, determine the number of SLNs, differentiate SLNs from subsequent nodes, locate the SLN in an unexpected location, and mark the SLN over the skin for biopsy. Single-photon emission computed tomography/computed tomography (SPECT/CT) has shown important benefits compared to planar lymphoscintigraphy in sentinel lymph node mapping.[66] The three-dimensional reconstruction images are a helpful tool, providing a simple yet comprehensive overview of the localization of hot spots. This type of image fusion provides better anatomical benchmarks, provides schematic information about the sentinel node site, and (perhaps most importantly) is easy to understand for surgeons, medi-

cal staff, and patients.[66] Nevertheless, conventional lymphoscintigraphy currently remains the only technique available to visualize the dynamic process of lymphatic drainage. Therefore SPECT/CT does not replace the conventional planar images; it can be considered as a complementary modality.

MRI of the brain

Although accuracy of FDG-PET/CT for detecting melanoma metastases is higher than MRI in nodal, cutaneous, subcutaneous, and pulmonary distant metastases staging,[67] the high physiological uptake of FDG in the normal brain limits the sensitivity for detecting brain metastasis, which is a frequent metastasis site in patients with melanoma.[68] MRI is the current gold standard for this purpose.[69] The necessity of performing routine brain imaging in asymptomatic patients with advanced locoregional disease is controversial. Some clinicians perform the procedure only in symptomatic patients to rule out central nervous system involvement, while others recommend brain imaging before definitive local therapy in accordance with a report showing a rate of asymptomatic central nervous system metastases as high as 6%.[70] MRI of the brain in asymptomatic stage I and II melanoma patients shows low detection rates and (as to be expected) a high frequency of false-positives and is therefore not recommended in these patients. Although early detection of brain metastases may identify a limited number of patients who are eligible for more aggressive local therapies, no available data demonstrate that screening for brain metastases results in a survival benefit for patients. A recent study showed that MRI detected lesions suspicious for melanoma metastases in only 1.6% of stage III melanoma patients.[68] Furthermore, two other recent studies showed no brain metastases detected by MRI in stage III melanoma patients.[71,72] Therefore routine MRI of the brain does not seem advisable for stage III melanoma patients. Stage IV melanoma patients should be evaluated with MRI of the brain because the likelihood of detecting additional asymptomatic lesions is high and management of stage IV patients can change due to the detection of brain metastases in these patients.[59] Further improvement in detecting melanoma metastases might be achieved by combining molecular, functional, and anatomical imaging using PET/MRI. It is expected to become an important tool for the detection of both lymph node and distant melanoma metastases.[73] However, data on the performance of PET/MRI are awaited but not available at present for melanoma.

Outline of the thesis

Part I Pathology of primary cutaneous melanoma

Pathologic parameters of the primary tumor are the strongest predictors of outcome in patients with clinically localized primary melanoma. Hence, an accurate and complete pathology report that documents all such features is essential for guiding the patient's initial treatment. Although evidence-based criteria have been established for the pathologic diagnosis and staging of melanoma, these are, to some extent, subjective parameters and there can be significant interobserver variation between pathologists in their assessment. Chapter 2 describes the interobserver variation between pathologists for

pathologic staging and diagnosis of melanoma. In addition, completeness of these pathologic reports is analyzed. Chapter 3 depicts how often diagnosis, and therefore treatment, changes due to interobserver variation between pathologists. In this chapter the need for review of an expert pathologist in the pathologic diagnosis and staging of melanoma is analyzed. Chapter 4 analyzes a pathologic feature that is not very common in melanoma but highly important; microsatellites. Melanoma patients with microsatellites have similar prognosis compared to melanoma patients with clinically evident satellites or intransit metastases. In this chapter the clinical implications of microsatellites are described and a new definition for this pathologic feature is proposed.

Part II Diagnosis and management of melanoma patients with lymph node metastases

The last part of this thesis focuses on diagnosis and treatment of melanoma patients with metastatic disease in the lymph nodes. A systematic review is performed in chapter 5 to define the optimal technique for SLNB. SLNB can be performed in the head and neck, the axilla, and in the groin. Melanoma patients with lymph node metastases can be treated with a lymph node dissection. A lymph node dissection of the groin can be performed in only the superficial (inguinal) or in both the superficial and the deep (iliac and obturator) area. Chapter 6 describes the importance of performing a combined superficial and deep lymph node dissection and the prognostic significance of deep lymph node metastases. Besides metastases in the deep lymph nodes, other prognostic factors for stage III patients have been suggested. One of these factors is the presence of a BRAF mutation, chapter 7 describes the prognostic significance of this mutation and discusses the use of systemic therapy in stage III melanoma patients with this mutation.

Part III Imaging modalities in melanoma

Chapter 8 describes the value of nuclear imaging modalities for all stages of melanoma. The additional value of FDG-PET/CT above other imaging modalities is explored as well as the limitations of FDG-PET/CT. Finally, future directions for nuclear imaging in melanoma are outlined in this chapter. The additional value of FDG-PET/CT in clinical stage III melanoma patients will be further outlined in chapter 9, where survival and recurrence of FDG-PET/CT positive and negative clinical stage III melanoma patients are analyzed.

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Part I

Pathology of melanoma

Chapter 2

Reproducibility of AJCC staging parameters in primary cutaneous melanoma: an analysis of 4924 cases

Maarten G. Niebling, Lauren Haydu, Rooshdiya Z. Karim, John F. Thompson, Richard A. Scolyer

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Abstract

Background

Pathology reports are critical for conveying information to clinicians who must make important management decisions for their patients. This study sought to assess and compare the precision, reproducibility, and completeness of external pathology reports and pathology reports generated by central review of each case in a large cohort of primary cutaneous melanoma patients.

Methods

Details of matched external pathology reports and corresponding review reports for 4924 primary cutaneous invasive melanomas diagnosed and treated at Melanoma Institute Australia (MIA) between 2001 and 2011 were analyzed.

Results

Interobserver agreement was excellent for American Joint Committee on Cancer (AJCC) T staging parameters: Breslow thickness (Intraclass Correlation Coefficient (ICC) 0.984), mitotic rate (ICC 0.833) and ulceration (Kappa statistic (κ) 0.823). All three of these important pathologic variables were included in 92.4% and 66.9% of review (MIA) and external (non-MIA) pathology reports, respectively. Completeness of MIA and non-MIA pathology reports for the three essential T-staging criteria increased significantly from 87.9% to 94.6% (χ^2=9.1, df1, P=0.003) and from 53.2% to 74.3% (χ^2=35.0, df1, P<0.001) over the ten-year study period. The AJCC N staging parameter microsatellites was recorded in only 43% of non-MIA reports and showed moderate concordance (κ=0.560).

Conclusions

Reproducibility and completeness of pathology reports for many important histopathologic features have improved in recent years. Nevertheless, the documentation of microsatellites remained poor in external pathology reports. To enhance the usefulness of the pathology report for the provision of optimal melanoma patient care continued efforts to encourage pathologists to document the key features appear warranted.

Introduction

Melanoma is a major public health problem in many Western countries. In Australia, melanoma is the third most common cancer in both men and women.[1] Accurate diagnosis and appropriate treatment at an early clinical stage is associated with high cure rates.[2] Pathologic parameters of the primary tumor are the strongest predictors of outcome in patients with clinically localized primary melanoma.[2] Hence, an accurate and complete pathology report that documents all such features is essential for guiding the patient's initial treatment.

Guidelines for the classification and staging of melanoma were updated by the American Joint Committee on Cancer (AJCC) in 2009.[3] Breslow thickness, ulceration and tumor mitotic rate (TMR) are the three essential T-staging criteria. Clark level of invasion is no longer included as a primary staging criterion for T1 melanomas, as its independent prognostic value is not as strong when the other factors are included in multivariate analysis. However, it can still be substituted as a criterion if tumor mitotic rate cannot be determined.[3] The presence of (micro)satellites is used in the N-classification of melanoma and therefore it is essential that this pathologic feature is also included in pathology reports.[3]

Although evidence-based criteria have been established for the pathologic staging of melanoma, these are, to some extent, subjective parameters and there can be significant interobserver variation between pathologists in their assessment. This is superimposed on the actual diagnosis of melanoma, which can itself be difficult and subjective.[4-7] Apart from the level of agreement between pathologists for various pathologic parameters, another related issue is the importance of documenting all the important pathologic parameters in histopathology reports.

At MIA, external pathology slides and reports are routinely reviewed by MIA-affiliated pathologists in the department of Tissue Pathology and Diagnostic Oncology at the Royal Prince Alfred Hospital (RPAH), Sydney, both to confirm the diagnosis and to document pathological prognostic parameters. There are therefore a large number of cases for which an outside report and subsequent internal review report are available for the same primary tumor. In this study, a retrospective analysis was performed on 4924 initial pathology reports and the corresponding subsequent review pathology reports. The primary aim of the study was to investigate the interobserver variation between community-based pathologists and Melanoma Institute Australia (MIA) pathologists. Secondary aims were to evaluate the completeness of the histopathology reports by comparing those from external pathologists with those issued by MIA pathologists, and to determine how reporting standards varied over time.

Methods

A subset of melanoma patients treated at MIA are diagnosed with primary melanoma elsewhere and subsequently referred to MIA for further management. In such cases, the original pathologic slides are

reviewed by MIA-affiliated pathologists of the Department of Tissue Pathology and Diagnostic Oncology at the RPAH (MIA pathologists), Sydney, Australia. Subsequent investigations and treatment of these melanoma patients are usually performed at MIA. For these patients, there are two pathology reports on the one melanoma specimen: one is generated by the original reporting (non-MIA) pathologist and the other by an MIA pathologist. In most instances, the MIA pathologist has a copy of the original pathology report when performing the pathology slide review. For this study, pathology reports were reviewed for patients who presented to MIA with a primary cutaneous melanoma between January 2001 and February 2011. Only cases where both MIA and non-MIA pathologists diagnosed invasive melanoma were selected (n=4924). Cases were analyzed for variations in the reporting of the following pathologic features: Breslow thickness (measured in mm) as a continuous variable and categorized in the four T-classification groups, TMR (measured in mitoses per mm^2) as a continuous variable and as a categorical variable (present or absent), Clark level of invasion (I-V), the presence or absence of ulceration, microsatellites, vascular invasion and lymphatic invasion; the association with a nevus; regression recorded as absent or present; and melanoma subtype categorized as superficial spreading melanoma (SSM), nodular melanoma (NM), acral lentiginous melanoma, desmoplastic melanoma (with or without neurotropism), lentigo maligna melanoma, other and unclassified. The completeness of pathology reports for all essential pathologic features (defined as those criteria necessary to stage the patient according to the 2009 version of the AJCC melanoma staging system i.e. tumor thickness, ulceration, TMR and microsatellites and whether or not the surgical margins were involved) and desirable pathologic features (the remaining features listed above) was also analyzed.

Statistical analyses

The interobserver variability for all categorical pathologic features was assessed with Cohen's Kappa statistic. For continuous pathologic features such as Breslow thickness and TMR, intraclass correlation coefficients (ICCs) were calculated to assess agreement. Values for ICC greater than 0.75 are considered to represent excellent agreement, values between 0.75 and 0.40 good to fair agreement, and values below 0.40 poor agreement between different observers. Kappa statistics for excellent agreement are between 0.81 and 1.00, values between 0.61-0.80 represent good agreement, between 0.41-0.60 moderate agreement, between 0.21-0.40 fair agreement and below 0.20 is considered to be poor agreement between observers.[14,15] The Chi square statistic was used to test if the difference in completeness of pathologic features between outside and review pathology reports was significant to a level of $p<0.05$. Univariate binary logistic regression was performed to test if disagreement for each pathologic feature was significantly associated with patient or primary melanoma staging characteristics.

Results

Interobserver variation

There were 4924 melanoma patients in whom both the MIA and non-MIA pathologist diagnosed invasive melanoma. The patients' median age was 64 years; 2019 were female (41.0%) and 2905 (59.0%) were male. The levels of agreement between the MIA and non-MIA pathologists for each pathologic feature are presented in Table 1.

Table 1. Agreement between non-MIA and MIA pathologists on pathologic features

Pathologic feature	Statistical test	Valid cases	Agreement (95% CI)	Strength of agreement
Breslow thickness	ICC	4785	0.984 (0.983-0.985)	Excellent
	Kappa	4785	0.860 (0.848-0.872)	Excellent
Tumor mitotic rate	ICC	3698	0.833 (0.822-0.843)	Excellent
	Kappa	3698	0.682 (0.654-0.710)	Good
Ulceration	Kappa	3743	0.823 (0.812-0.834)	Excellent
Clark	Kappa	4567	0.627 (0.617-0.637)	Good
Microsatellites	Kappa	1978	0.560 (0.514-0.606)	Moderate
Vascular invasion	Kappa	3644	0.440 (0.395-0.485)	Moderate
Lymphatic invasion	Kappa	2045	0.565 (0.514-0.616)	Moderate
Associated nevus	Kappa	2421	0.551 (0.537-0.565)	Moderate
Regression	Kappa	2780	0.236 (0.226-0.249)	Fair
Melanoma subtype	Kappa	3381	0.588 (0.577-0.599)	Moderate

ICC: Intraclass correlation coefficient; CI: Confidence interval.

The ICC for Breslow thickness was 0.984, indicating excellent overall concordance. Concordance for Breslow thickness as a categorical variable was also excellent (κ=0.860) Both MIA and non-MIA pathologists reported Breslow thickness in 4785 cases; both pathologists agreed on Breslow thickness in 1837 of those cases (38.4%) and in 1521 cases (31.8%) the disagreement was ≤0.1mm. However, in 1086 cases (22.7%) the disagreement was 0.1-0.5 mm and in 340 cases (7.1%) the difference was >0.5mm (Figure 3).

The ICC for TMR was 0.833, indicating excellent concordance for this parameter also. Concordance on the presence or absence of TMR was good (κ=0.682). Pathologists agreed on the presence or absence of TMR in 3276 cases (88.6%). TMR changed in 305 cases (8.2%) from absent to present and in 118 cases (3.2%) from present to absent after review. In 1520 cases (41.1%) pathologists agreed on the exact number of mitoses per square millimeter. Where the pathologists disagreed on the number of mitoses, in 1527 cases (41.3%) the difference was ≤3 mitoses/mm^2, and in 651 cases (17.6%) the difference was >3 mitoses/mm^2.

Concordance was excellent for ulceration (κ=0.823); good for Clark level of invasion (κ=0.627) and melanoma subtype (κ=0.620); moderate for microsatellites (κ=0.560), vascular invasion (κ=0.440), lymphatic invasion (κ=0.565), and associated nevus (κ=0.551); and fair for regression (κ=0.236). Figure 1 shows the interobserver agreement each year for 5 important pathologic features over the period 2001-2010.

There were no systematic differences in the direction of disagreement for any of the pathologic parameters in cases where the MIA pathologists disagreed with the non-MIA pathology reports (i.e. there was no tendency to "overcall" or "undercall").

Neither the age or sex of the patient were associated with the level of agreement between pathologists for any of the pathologic features analyzed in the present study. However, disagreement between pathologists in the reporting of Breslow thickness (OR=1.173, 95%CI: 1.11-1.25, p<0.001), TMR (OR=1.50, 95%CI: 1.41-1.59, p<0.001), and ulceration (OR=1.08, 95%CI: 1.04-1.13, P<0.001) was significantly more common in thicker tumors. Disagreement regarding Clark level of invasion was more common in thinner tumors (OR= 0.89, 95%CI: 0.85-0.93, P<0.001). Finally, disagreement on the TMR was more likely in cases where ulceration was present (OR= 3.25, 95%CI: 2.67-3.95, P<0.001) and disagreement on ulceration was more likely in cases where TMR was present (OR=2.95, 95%CI: 1.89-4.61, P<0.001).

Table 2. Frequency with which pathologic parameters were recorded in MIA and non-MIA pathology reports of invasive melanomas

Pathologic feature	**Frequency of recording of variable in pathology reports**		
	MIA (n=4924)	Non-MIA (n=4924)	Significance
Breslow thickness	99.6%	97.3%	χ^2:77.70, df1, P<0.001
Tumor mitotic rate	95.2%	77.5%	χ^2:428.98, df1, P<0.001
Ulceration	95.1%	78.3%	χ^2:363.76, df1, P<0.001
Clark	98.6%	93.4%	χ^2:104.64, df1, P<0.001
Microsatellites	87.6%	43.0%	χ^2:1274.94, df1, P<0.001
Vascular invasion	91.7%	78.3%	χ^2:126.79, df1, P<0.001
Lymphatic invasion	88.9%	45.0%	χ^2:1455.90, df1, P<0.001
Associated nevus	90.2%	51.5%	χ^2:1042.10, df1, P<0.001
Regression	91.6%	59.7%	χ^2:883.30, df1, P<0.001
Melanoma subtype	86.0%	74.5%	χ^2:1.02, df1, P=0.314

elanoma Institute Australia. Non-MIA: reports of pathologists outside the MIA.

χ^2: Chi square Df: Degrees of freedom

Completeness of pathology reports

Of all 4924 cases, 4555 MIA pathology reports (92.5%) and 3296 non-MIA pathology reports (66.9%) contained details of the three essential T-staging pathologic features: Breslow thickness, TMR and ulceration. Breslow thickness and Clark level of invasion were the most frequently reported pathologic features. Breslow thickness was reported in 99.6% of the MIA pathology reports and 97.3% in non-MIA pathology reports (Table 2). Clark level of invasion was reported in 98.6% and 93.4%, TMR in 95.2% and 77.5%, and ulceration in 95.1% and 78.3% of MIA and non-MIA pathology reports, respectively. Results of completeness for the remaining desirable pathologic features are presented in Table 2.

Table 3. Frequency of recording pathologic features for MIA and non-MIA pathologists for the years 2001 and 2010

	MIA			non-MIA		
	2001	2010	Statistics	2001	2010	statistics
Thickness	99.72%	100%	$\chi^2$7.34, df9, P=0.602	97.11%	98.05%	$\chi^2$7.45, df9, P=0.591
TMR	90.77%	97.28%	$\chi^2$50.92, df9, P<0.001	68.32%	82.88%	$\chi^2$63.79, df9, P<0.001
Ulceration	94.35%	95.33%	$\chi^2$8.72, df9, P=0.464	68.73%	84.44%	$\chi^2$63.79, df9, P<0.001
Clark	98.35%	98.83%	$\chi^2$12.65, df9, P=0.179	92.01%	92.61%	$\chi^2$12.19, df9, P=0.203
Microsatellites	84.30%	86.38%	$\chi^2$15.37, df9, P=0.08	26.39%	59.14%	$\chi^2$93.66, df9, P<0.001
Vascular invasion	92.84%	90.27%	$\chi^2$10.80, df9, P=0.290	67.22%	87.55%	$\chi^2$323.25, df9, P<0.001
Lymphatic invasion	88.84%	87.55%	$\chi^2$6.78, df9, P=0.660	28.65%	56.81%	$\chi^2$134.57, df9, P<0.001
Associated nevus	89.12%	89.88%	$\chi^2$5.38, df9, P=0.800	38.71%	61.48%	$\chi^2$160.39, df9, P<0.001
Regression	92.29%	89.49%	$\chi^2$10.06, df9, P=0.345	52.07%	66.54%	$\chi^2$97.23, df9, P<0.001
Melanoma subtype	83.61%	88.72%	$\chi^2$12.90, df9, P=0.167	73.55%	74.32%	$\chi^2$17.01, df9, P=0.047

MIA: Melanoma Institute Australia. Non-MIA: reports of pathologists outside the MIA.

χ^2: Chi square

Df: Degrees of freedom

Each of the 10 parameters were recorded significantly more frequently in MIA pathology reports than in non-MIA reports ($p<0.001$ for all parameters), except for melanoma subtype ($p=0.314$). Figure 2 shows the frequency with which the five key parameters were included in MIA and non-MIA pathology reports each year for the period 2001-2010. A comparison of the frequency of recording various pathologic features in the years 2001 and 2010 by MIA and non-MIA pathologists is presented in Table 3. Completeness of MIA and non-MIA pathology reports for the three essential T-staging criteria increased significantly from 87.9% to 94.6% ($\chi^2=9.1$, df 1, $P=0.003$) and from 53.2% to 74.3% ($\chi^2=35.0$, df 1, $P<0.001$) over the ten-year study period.

Over the time period of the study, there was a significant increase in the utilization of a synoptic/structured pathology-reporting format, particularly by non-MIA pathologists. Of the pathology reports in the year 2001, 92.3% of MIA and 28.1% of non-MIA reports had a synoptic format. In the year 2010, 98.2% of MIA reports and 62.4% of non-MIA reports had a synoptic format. The increase in utilization of synoptic reports was significant both for MIA reports (X^2 10.85, df 1, $P=0.001$) and for non-MIA reports (X^2 392.29, df 1, $P<0.001$).

Discussion

The prognosis for a patient with clinically localized primary cutaneous melanoma is principally determined by pathologic parameters of their primary tumor. Furthermore, these parameters are utilized for guiding the next stages of the patient's management such as the width of excision margins at the primary tumor site, the appropriateness of sentinel lymph node biopsy and, in some cases, eligibility for clinical trials of adjuvant therapies. It is therefore essential that the pathology report accurately documents these important parameters.

Concordance of Pathology Reports

Several studies have investigated the interobserver variability between pathologists for their assessment of various pathologic features.[19-31] These studies have shown that Breslow thickness is the feature with the highest concordance between pathologists. The presence or absence of ulceration has also shown high concordance. The present study showed excellent agreement between pathologists on TMR when this is analyzed as a continuous variable. However, concordance on the presence or absence of TMR was lower, although still good. It seems that although the disagreement incrementally in TMR is minor, it is actually occurring at the threshold that is important for staging. Studies on the concordance of TMR have produced different results. However, in a study published in 2003, Scolyer et al demonstrated excellent interobserver reproducibility amongst pathologists with widely differing experiences in the reporting of melanocytic tumors, utilizing a defined methodology for TMR assessment. This methodology is recommended for TMR assessment and reporting in the current version of

AJCC Staging System. Some other pathologic features have lower levels of concordance, such as Clark level of invasion.[19-31] Figure 1 shows improvement of reproducibility of ulceration, TMR, Clark level, and microsatellites over the ten-year study period.

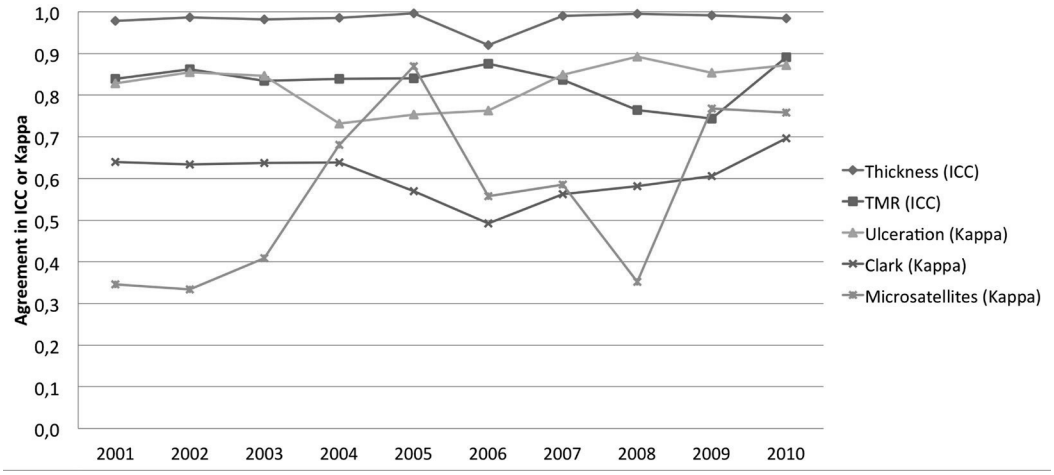


Figure 1. Interobserver agreement (Intraclass Correlation Coefficient or Kappa score) for 5 important pathologic features for the period 2001-2010

Microsatellites are associated with an increased frequency of lymph node metastasis and several studies have shown that the survival of patients with microsatellites is comparable with that of patients with clinically detectable satellite metastases.[32-36] This pathologic feature is used in the AJCC category of N2c melanoma.[3] Microsatellites are defined by the AJCC as 'any discontinuous nest of metastatic cells more than 0.05mm in diameter that are clearly separated by normal dermis (not fibrosis or inflammation) from the main invasive component of melanoma by a distance of at least 0.3 mm'.[3] We are not aware of any convincing evidence to support the use of this definition and because microsatellites are likely to represent local metastases we suggest a more appropriate definition to be 'any nest of metastatic tumor cells discontinuous from the primary tumor (but not separated only by inflammation or fibrosis)'. Microsatellites were not commonly seen, and were reported as present in only 148 (2.3%) of review reports. There was only moderate agreement between outside and review pathologists in the reporting of this parameter (κ=0.560). To the best of our knowledge there are no other studies reported in the literature to date that have calculated the agreement between pathologists on microsatellites with Cohen's Kappa statistic. In our view, further studies assessing the prognostic significance of microsatellites are warranted.

A potential weakness of the present study is that, for most reviews, MIA pathologists had access to the prior (non-MIA) pathology report, potentially influencing the MIA report. Therefore some MIA

pathology reports may not have been independent unbiased observations of the same case and this should be considered when interpreting our results.

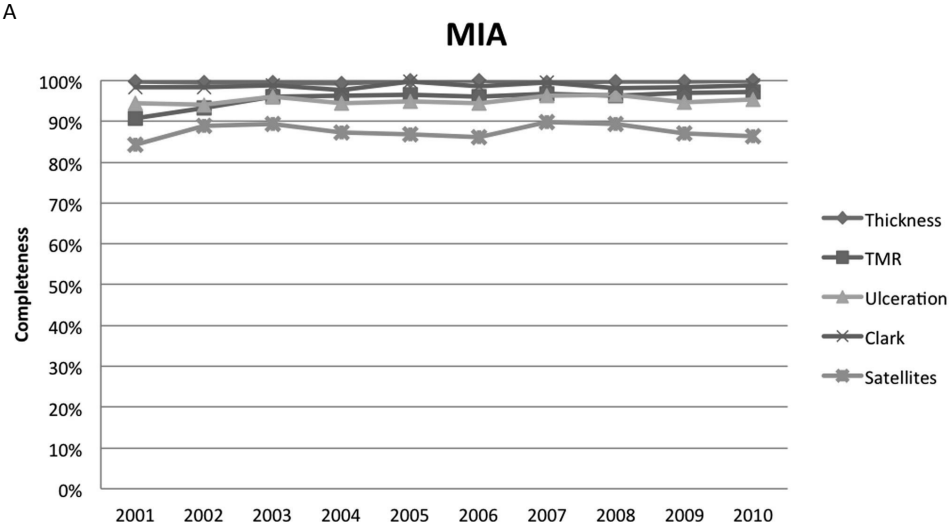
Completeness of Pathology Reports

For accurate determination of prognosis and for guiding appropriate management, it is essential that key pathologic features are documented in the melanoma pathology report. Previous studies have highlighted the fact that in many instances this is suboptimal.[8-10] Completeness of pathology reports ranged from 17% to 48% for the key pathologic features in staging melanoma.[8-11] In the present study completeness of the three essential pathologic T-staging criteria was higher in MIA (92.4%) and non-MIA (66.9%) reports. Moreover, completeness for these pathologic features improved significantly over the ten-year study period for both MIA reports (χ^2=35.0, df 1, P<0.001) and non-MIA (χ^2=9.1, df 1, P=0.003). In previous studies, Breslow thickness and Clark level of invasion were the best recorded pathologic features.[8-11] Ulceration was generally not well recorded and the recording of TMR and microsatellites were even less satisfactory.[8-11] As it is only comparatively recently that the prognostic importance of TMR has been highlighted in the literature and because it was only included as a staging criterion in the 2009 version of the AJCC staging system, the relatively poor documentation of TMR in melanoma pathology reports suggests that many pathologists are not aware of this recent literature. Similarly, as microsatellites are an N staging (rather than T staging) criterion, pathologists may not be aware of its importance for accurate patient staging. Continued efforts to educate the pathology community of the importance of documenting the key pathologic variables would appear to be necessary.

In our study, MIA pathology reports documented all features more frequently than non-MIA pathology reports (Table 3 and Fig. 2). However, there was a marked and significant improvement in the completeness of non-MIA pathology reports over the ten-year study period. All ten pathological parameters were reported more frequently in non-MIA reports in 2010 compared with 2001 and these improvements were statistically significant for all parameters except tumor thickness and Clark level. In contrast, MIA pathology reports documented all features at a high frequency throughout the study period and whilst there were some minor variations, the only significant change was more frequent reporting of TMR in 2010.

Four prior studies have reported findings on the frequency of reporting of pathologic variables and two of them also showed that completeness of reports increases with the use of a synoptic format (Table 4).[8-11] Importantly, in our study the frequency with which pathology reports were in a synoptic format increased for both MIA and non-MIA pathology reports over the study period, especially for non-MIA reports (28.1% in synoptic format in 2001 versus 62.4% in 2010), and this coincided with an increase in the frequency with which many of the pathologic variables were reported.

A



B

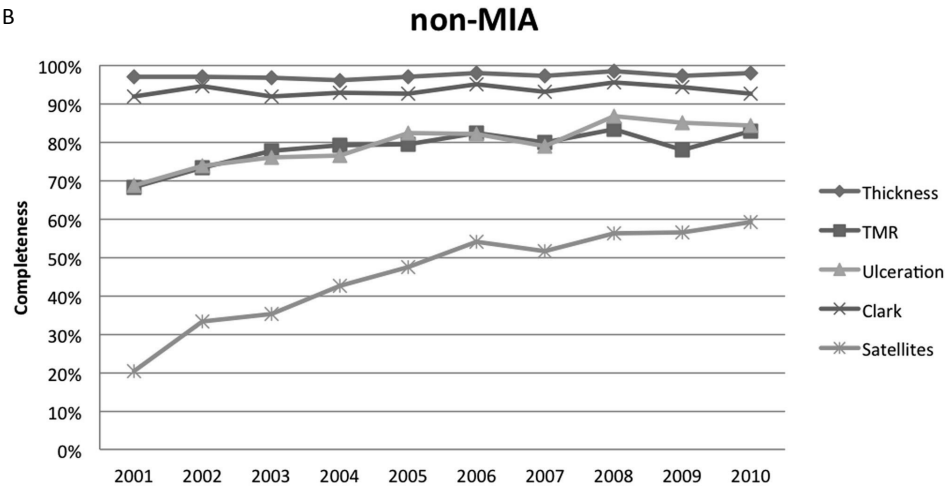


Figure 2. Frequency of recording of 5 important pathologic features between 2001 and 2010 in **A:** MIA pathology reports. **B:** non-MIA pathology reports.

The development, implementation and widespread use of the recently published RCPA structured melanoma pathology reporting protocol in Australia is likely to further improve the completeness and quality of melanoma pathology reports in the future. It has even been suggested that adherence to these guidelines in Australia may become necessary for laboratory accreditation, which in turn has implications for eligibility to receive government funding/reimbursement for reporting pathology specimens. Furthermore, the efforts of the international pathology community (through the respec-

tive pathology colleges of the USA, Canada, the United Kingdom and Australasia) to develop agreed uniform melanoma pathology reporting guidelines for implementation in each of their respective jurisdictions are likely to assist in this effort internationally.[37] Nevertheless, validation of success of these efforts in the future will be necessary to justify the considerable continued resource commitment that this entails.

Table 4. An example of a synoptic structured pathology report for a primary cutaneous melanoma

Pathological feature	**Example**
Sex	Female
Site	Right leg
Diagnosis	Melanoma
Histological subtype	Superficial spreading melanoma
Vertical growth phase	Present
Breslow thickness	2.6mm
Ulceration (diameter in mm)	Present (3.3mm)
Dermal mitotic index (per mm^2)	4 per mm^2
Clark level	IV
Vascular or lymphatic invasion	Absent
Neurotropism	Present
Desmoplasia (% of dermal invasive tumor)	Absent
Satellites	Absent
Features or regression	
Early (TILs)	Mild and focal (non-brisk)
Intermediate (angiofibroplasia ± TILs)	Absent
Late (fibrosis and loss of rete ridges)	Absent
Predominant cell type	Epithelioid
Associated nevus	Dysplastic compound nevus
Nearest lateral margin to *insitu* component	1.4mm
Nearest lateral margin to dermal invasive component	3.2mm
Distance from tumor to deep margin	5.3mm
Solar elastosis	Mild (1+)

TIL: tumor-infiltrating lymphocyte

In conclusion, the present study shows improvement in the quality and completeness of melanoma pathology reporting over the ten-year study period which is probably due to the more frequent utilization of synoptic pathology reports. Nevertheless, in the final year of the study (2010) the three essential T-staging criteria were still only documented in 74% of external pathology reports and N-staging parameter microsatellites in only 59%. Therefore, further efforts to encourage pathologists to document the essential histopathologic features in all melanoma reports appear warranted which should enhance their usefulness as tools for assisting in the provision of optimal patient care.

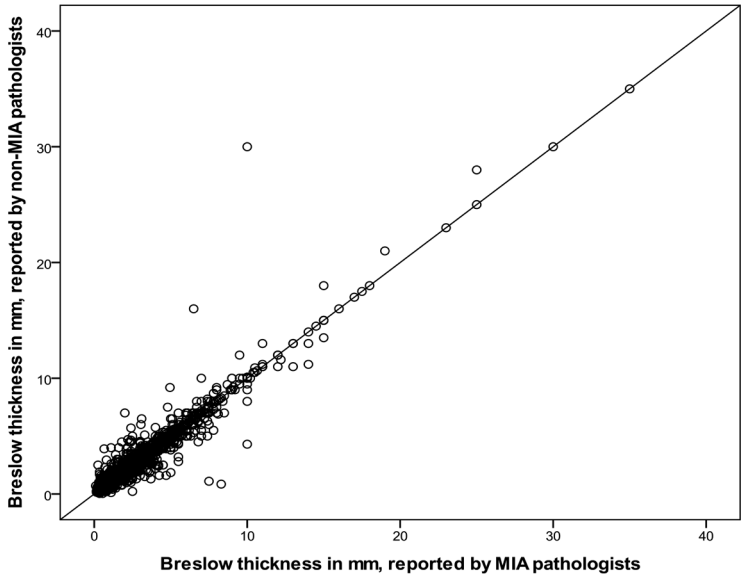


Figure 3. Scatterplot demonstrating interobserver agreement of all valid cases (n= 4785) for Breslow thickness (mm)

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Chapter 3

Pathology review significantly impacts on diagnosis and management of melanoma patients: an analysis of 5011 patients treated at a melanoma treatment center

Maarten G. Niebling, Lauren Haydu, Rooshdiya Z. Karim, John F. Thompson, Richard A. Scolyer

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Abstract

Introduction

Pathologists sometimes disagree on the diagnosis of melanoma or its histopathologic staging, which may have implications for treatment and follow-up. For this reason, melanoma patients referred to Melanoma Institute Australia (MIA) for further management routinely have their pathology slides reviewed by MIA pathologists. This study sought to determine if diagnosis, staging and management of melanoma patients changed significantly following central pathology review.

Methods

5011 pairs of non-MIA and MIA pathology reports on the same primary melanoma specimen were reviewed. Differences in diagnosis, American Joint Committee on Cancer (AJCC) T-classification and management recommendations based on the non-MIA and MIA pathology reports were determined.

Results

A melanoma diagnosis changed in 5.1% of cases after review. Where both pathologists agreed on a diagnosis of melanoma, AJCC T-classification changed in 22.1% after review. After MIA review, planned surgical excision margins changed in 11.2% of cases and a recommendation for sentinel lymph node biopsy (SLNB) changed in 8.6%. Non-MIA reports less frequently contained criteria to define AJCC T-classification (86.6% versus 97.6%), select appropriate surgical excision margins (95.2% versus 99.6%) and make a recommendation for SLNB (94.5% versus 99.4%), (each $p<0.001$). On multivariate analysis, partial biopsies were independently associated with more frequent changes in AJCC T-classification ($p<0.001$), planned surgical excision margins ($p<0.001$) and SLNB recommendations ($p<0.001$) on the basis of MIA pathology review.

Conclusions

Diagnosis, AJCC T-classification and management recommendations often change following pathology review by specialist melanoma pathologists. We recommend pathology review be considered for all patients attending specialist melanoma treatment centers.

Introduction

For patients presenting with a primary cutaneous melanoma, an accurate and comprehensive histopathology report is essential for staging, to determine appropriate management and to provide a reliable estimate of prognosis[1-4]. Guidelines for the classification and staging of melanoma established in 2002[5] by the American Joint Committee on Cancer (AJCC) were updated in 2009[6]. Clinical guidelines for the management of melanoma patients have also been published[7-12]. The key pathologic factors for determining stage, prognosis and management are tumor thickness, mitotic rate, ulceration and the proximity of the tumor to the excision margins[5,6].

The histopathologic diagnosis and staging of melanoma can be challenging and remain subjective, with significant interobserver variability between pathologists[13-20]. Inaccurate staging of melanoma patients can result in inappropriate management, inaccurate prognosis and inadequate follow-up. Pathology review by expert melanoma pathologists is considered likely to improve not only the accuracy of melanoma pathology reports but also the frequency with which pathology reports document the key parameters essential for staging, prognosis and management[17,21-27]. For these reasons, patients referred to many major melanoma treatment centers for further management after a melanoma diagnosis has been made have their pathology slides reviewed and re-evaluated by treatment center pathologists. This study sought to determine if the diagnosis, AJCC T-classification, and management of melanoma patients significantly changed as a consequence of pathology review by melanoma treatment center-affiliated specialist pathologists.

Methods

At Melanoma Institute Australia (MIA), the external pathology slides and reports of patients referred for melanoma treatment are routinely reviewed by at least one (but usually two, and sometimes three) MIA-affiliated pathologists in the department of Tissue Pathology and Diagnostic Oncology at the Royal Prince Alfred Hospital, Sydney, both to confirm the diagnosis and to document pathologic prognostic parameters. There are therefore a large number of cases for which an outside report and a subsequent internal review report are available for the same primary tumor. For this study, patients referred from 2002 to 2011 were selected, and consecutive paired reports on the same specimens from non-MIA and MIA pathologists were identified (n=5011). Each case was categorized based on the diagnosis recorded in the report as benign, melanoma in situ or invasive melanoma. Thereafter, each case of invasive melanoma was assigned an AJCC T-classification. For the cases diagnosed between 2002 and 2008, T-classification was defined according to the 2002 AJCC criteria, dependent on Breslow thickness and ulceration, and for T1 melanomas also Clark level of invasion. For cases diagnosed between 2009 and 2011, the latest 2009 AJCC criteria were used for T-staging. For the 2009 AJCC staging system, T1 melanomas were first defined using tumor mitotic rate (TMR) and ulceration, and if those features were not reported, Clark level of invasion was used, as recommended by the AJCC.[6]

The appropriate surgical management for each case was based on the Breslow thickness of melanoma, as per the recommendations of the Australian and New Zealand Clinical Practice Guidelines for the Management of Melanoma[11]. These guidelines recommended that melanoma in situ cases be excised with a margin of 5mm; invasive melanomas with a thickness ≤1.00mm with a 1cm margin; melanomas 1.01mm - 4.00mm with a 1-2cm margin, depending on the anatomic location and clinical features; and melanomas ≥4.01mm with a 2cm margin. Whether or not sentinel lymph node biopsy (SLNB) would have been recommended based on management guidelines and the features documented in the pathology report (for AJCC T1b or higher) was also considered[12].

The completeness of both non-MIA and MIA pathology reports for the histopathologic criteria utilized to define AJCC T-classification, select surgical excision margins and make a recommendation for SLNB was also analyzed.

Statistical analysis

The chi square statistic (χ^2) was used to test whether the difference in completeness of pathology reports regarding pathologic features necessary for determining AJCC T classification, appropriate surgical excision margins and the need for SLNB between non-MIA and MIA pathologists was significant to a level of $p<0.05$. Univariate and multivariate logistic regression analyses were performed to determine which patient and melanoma factors were associated with a change in diagnosis, AJCC T-classification or surgical management.

Results

5011 pairs of non-MIA and MIA pathology reports were compared (2031 female (40.5%), 2980 males (59.5%), median age 65 years).

Diagnosis

Non-MIA and MIA pathologists had discordant diagnoses in 255 of the 5011 cases (5.1%). Of the 529 cases diagnosed as melanoma in situ by non-MIA pathologists, 108 (20.4%) were upgraded to invasive melanoma and 8 (1.5%) were downgraded to benign lesions on review. Of the 3753 cases diagnosed as invasive melanoma by non-MIA pathologists, 14 (0.4%) were downgraded to benign and 125 (3.3%) were downgraded to melanoma in situ on review (Table 1).

T-classification

Of all 5011 non-MIA and MIA pathology reports, 4338 non-MIA pathology reports (86.6%) and 4888 MIA pathology reports (97.6%) contained the essential criteria for determining AJCC T-classification. The completeness of non-MIA reports for AJCC T-classification was significantly lower than that of MIA reports (χ^2: 156, df 1, $P<0.001$) (Table 5).

Table 1. Number of cases with a change in staging after review by MIA pathologists

		T-classification after review											
		Benign	Tis	T1a	T1b	T2a	T2b	T3a	T3b	T4a	T4b	Unknown*	Total
	Tis	8	416	97	5	0	0	3	0	0	0	3	532
	T1a	9	107	988	110	40	1	2	0	0	0	8	1265
	T1b	2	7	140	180	44	6	0	0	0	0	4	383
T-	T2a	0	7	46	21	716	8	30	1	1	0	21	851
classification	T2b	0	1	3	5	57	144	5	16	0	0	6	237
before	T3a	0	0	4	1	31	1	323	15	7	0	7	389
review	T3b	0	0	0	0	4	9	42	276	1	13	2	347
	T4a	0	0	0	0	0	0	13	2	105	4	5	129
	T4b	0	0	0	0	0	0	2	10	17	176	0	205
	Unknown*	3	3	19	113	42	154	18	142	23	69	67	673
	Total	22	561	1391	364	1046	187	559	343	199	216	123	5011

*Invasive melanoma cases where the non-MIA or MIA pathology report did not contain the essential criteria for defining T-classification.

Of the cases where both non-MIA and MIA pathologists agreed on a diagnosis of melanoma (n=4269), T-classification changed in 945 cases (22.1%). Both non-MIA and MIA pathologists agreed on the diagnosis of invasive melanoma in 3620 cases. In 712 of these cases (19.7%), the T-classification was changed on review; in 304 cases (8.4%) the T-classification was higher and in 408 (11.3%) it was lower after review (Table 1). Figure 1 shows the percentage of cases with a change in diagnosis or T-classification in each year from 2002 to 2010.

Surgical margin recommendations

The essential criteria for determining appropriate surgical excision margins were less frequently reported in non-MIA (4770 cases, 95.2%) than in MIA (4990 cases, 99.6%) pathology reports (χ^2: 120, df 1, P<0.001) (Table 1).

Both the non-MIA and the MIA pathology reports contained the essential criteria for defining appropriate surgical excision margins in 4759 cases. Planned margins changed after MIA review in 531 cases (11.2%); they changed to a wider excision in 236 cases (5.0%), and a narrower excision in 295 cases (6.2%) (Table 2). Figure 1 shows the percentage of cases for each year with a change in recommended surgical excision margins from 2002 to 2010.

Table 2. Number of cases with a change in surgical margin recommendation after review by MIA pathologists

		Recommended excision margins after review						
		conservative excision	0.5cm	1cm	1-2cm	2cm	unknown*	Total
	0.5cm	8	416	103	4	0	1	532
Recommended excision	1cm	12	116	1449	100	0	6	1683
margins	1-2cm	1	16	108	2005	29	3	2161
before review	2cm	0	0	0	32	361	1	394
	unknown*	1	11	106	72	41	10	241
	Total	22	561	1766	2212	429	21	5011

*Invasive melanoma cases where the non-MIA or MIA pathology report did not contain the essential criteria for defining surgical excision margins.

Sentinel lymph node biopsy recommendations

The essential criteria for defining the appropriateness of a SLNB were reported less frequently in non-MIA (n=4735, 94.5%) than MIA (n=4979, 99.4%) reports, respectively (χ^2: 144, df 1, P<0.001) (Table 1). These essential criteria were provided by both non-MIA and MIA pathologists in 4719 cases. The changes in whether or not SLNB would have been offered after MIA review are presented in Table 3. The recommendation for SLNB changed after MIA review in 407 patients (8.6%). SLNB would have been offered in 239 patients (5.1%) whereas in 168 cases (3.5%) it would not have been recommended.[12] The percentages of cases for each year between 2002 and2010 with a change in the SLNB recommendation after review by MIA pathologists are presented in Figure 1.

Table 3. Number of cases with a change in SLNB recommendation after MIA review

		After MIA review			
		no	yes	unknown*	total
	no	1626	168	9	1803
Before MIA review	yes	239	2686	7	2932
	unknown*	109	151	16	276
	total	1974	3005	32	5011

* Invasive melanoma cases where the non-MIA or MIA pathology report did not contain the essential criteria for defining recommendations for SLNB.

Factors influencing change in diagnosis, T-classification, or management

Table 4 shows the results of multivariable analysis of factors associated with a change in diagnosis, T-classification, surgical excision margin and recommendation for SLNB. Age and gender of the patient were not independently significantly associated with a more frequent change in diagnosis, T-classi-

fication, or surgical excision margins/SLNB recommendations. Melanomas with a Breslow thickness ≤1.00mm and melanomas located on the head or neck were independently associated with more frequent changes in diagnosis. Melanomas diagnosed by partial biopsy and melanomas located on the head or neck were independently associated with more frequent changes in T-classification. Melanomas with a Breslow thickness ≤1.00, melanomas diagnosed by partial biopsy, and melanomas located on the head or neck were independently associated with more frequent changes in recommended surgical excision margins. Finally , melanomas with a Breslow thickness ≤1.00mm, melanomas diagnosed by partial biopsy and melanomas located on the head or neck were independently associated with more frequent changes in recommendations for SLNB.

Discussion

Misdiagnosis or inaccurate reporting of a melanoma may result in inappropriate management, inadequate follow up and contribute to an adverse outcome. There may also be medicolegal consequences. However, the histopathologic diagnosis of melanoma can be difficult and review by a pathologist with special expertise in the assessment of melanocytic skin tumors may improve the accuracy of the diagnosis and the reporting of its important attributes.

Change in diagnosis

In this study, the diagnosis of the lesion changed in 5.1% of cases after review by an MIA pathologist. This figure is within the range (2.9% to 27%) reported in prior studies[13,15,20,23,24,26-28]. Fewer cases (4.3%) had a change in diagnosis in 2010 compared to 2002 (7.4%) (Figure 1). However, it must be noted that in some prior studies, in contrast to our study, cases that were referred for a second opinion due to uncertainty/difficulty with the diagnosis were also included.

The histopathologic diagnosis of melanoma in situ was an area of particular difficulty[26]. The diagnosis of melanoma in situ changed in 21.9% of cases; 20.4% of cases were upstaged to invasive melanoma and 1.5% of cases were downgraded to a benign lesion. Reasons for this included under-recognition of subtle dermal invasive melanoma (e.g. desmoplastic melanoma) by non-MIA pathologists, or the classification of dermal cells as representing an associated nevus rather than dermal invasive melanoma. Nevertheless, our results show the value of having in situ (as well as invasive) melanomas reviewed by expert pathologists.

Change in AJCC T-classification

Of the cases where both non-MIA and MIA pathologists agreed on a diagnosis of melanoma, T-classification changed in 22.1%, and where they agreed on the diagnosis of invasive melanoma, T-classification changed in 19.7%. AJCC classification T1b had the least agreement. Of 379 cases that non-MIA pathologists considered T1b, only 180 cases (47.5%) were classified as T1b after review. Classification T2b also had relatively low agreement; in only 62.3% of the cases did the pathologists agree

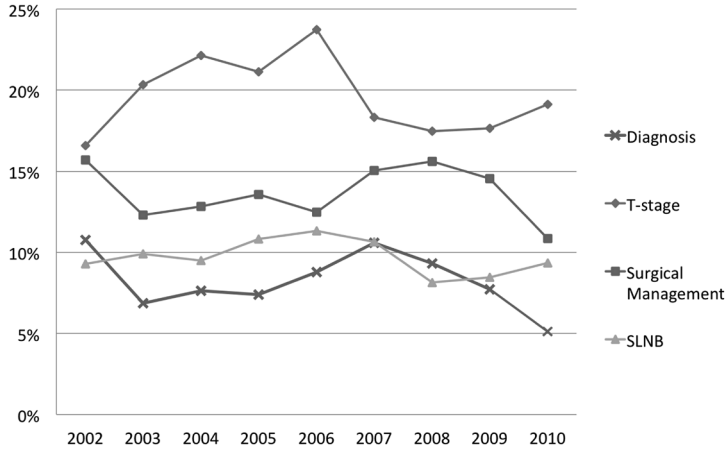

Figure 1. Percentages of cases with changes in diagnosis, T-classification and recommendations for surgical excision margins and SLNB after review by an MIA pathologist for each year from 2002-2010.

on this T-classification. For the other T-classifications, agreement between pathologists ranged from 78% to 86%. Low agreement in classification of T1b (55.3% of T1b cases were changed to T1a) was mostly due to the fact that Clark level of invasion was used for staging in the 2002 version of the AJCC staging system and, as we have previously documented, it has poorer interobserver reproducibility compared with other T-classification criteria[16,17,19]. The frequency of a change in T-classification was higher in 2010 (21.4%) compared to 2002 (16.6%) (Figure 1). In T2b cases, T-classification changed in 24.7% to T2a and in 12.2% changed to either T3a/b or T1a/b due to discordances in thickness. The more frequent staging discrepancy in T2b melanomas is difficult to explain since ulceration is a pathologic parameter with documented excellent concordance[13-20] and there were not many changes from T2a to T2b.

Change in recommended surgical excision margins

Appropriate surgical excision margins (as per the Australian and New Zealand management guidelines) changed in 11.2% of cases after review by an MIA pathologist. However, the percentage of cases with changes in recommended surgical excision margins fell substantially over the ten year period of this study. In 2002, 13.2% of patients would have had inappropriate surgical excision margins based on the non-MIA report, whereas in 2010 this was reduced to 8.4% of patients (Figure 1). Appropriate surgical excision margins for cases reported as melanoma in situ by non-MIA pathologists changed in 21.7% of cases. In 6.1% of cases the change in appropriate surgical excision margins was due to a change in T-classification, where the pathologists disagreed on Breslow thickness.

Table 4. Multivariable regression analysis for factors which were associated with change in diagnosis, T-classification, surgical excision margins and SLNB recommendations after review by MIA pathologists

		Diagnosis			T-classification			Surgical excision margins			SLNB		
		OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
Age^	0-52 years			NS			NS			NS			NS
	53-64 years			NS			NS			NS			NS
	65-75 years			NS			NS			NS			NS
	>75 years			NS			NS			NS			NS
Gender	Male			NS			NS			NS			NS
	Female			NS			NS			NS			NS
Breslow thickness	0-1.00mm	1					NS	1			1		
	1.01-2.00mm	0.19	0.13-0.28	**<0.001**			NS	0.51	0.41-0.63	**<0.001**	0.21	0.17-0.27	**<0.001**
	2.01-4.00mm	0.17	0.10-0.27	**<0.001**			NS	0.38	0.29-0.49	**<0.001**	0.15	0.11-0.21	**<0.001**
	>4.00mm	0.16	0.08-0.31	**<0.001**			NS	0.82	0.61-1.10	0.178	0.31	0.23-0.46	**<0.001**
Type of biopsy	Partial*			NS	1			1			1		
	Excision			NS	0.66	0.56-0.78	**<0.001**	0.69	0.56-0.86	**<0.001**	0.65	0.52-0.81	**<0.001**
Location of PM	Head & neck	1			1			1			1		NS
	Arm	0,56	0.37-0.86	**0.002**	0.83	0.67-1.02	0.080	0.67	0.51-0.88	**0.004**	0.86	0.64-1.14	0.297
	Trunk	0.57	0.41-0.81	**0.002**	0.74	0.62-0.88	**0.001**	0.61	0.48-0.77	**<0.001**	0.66	0.52-0.85	**0.001**
	Leg	0.53	0.35-0.79	**<0.001**	0.75	0.62-0.91	**0.006**	0.55	0.42-0.71	**<0.001**	0.73	0.55-0.96	**0.024**

^The four age groups represent the interquartile range. *An incision, shave or punch biopsy. SLNB: Sentinel Lymph Node Biopsy. OR: Odds Ratio. CI: Confidence Interval. NS: Not Significant. PM: Primary Melanoma

Changes in recommendations for sentinel lymph node biopsy

For AJCC staging and according to the evidence-based American Society of Clinical Oncology/Society of Surgical Oncology practice guideline, SLNB is today usually offered to patients with melanomas of classification T1b or higher[6,12,29,30]. A recent study of patients with in situ and thin invasive melanomas showed that in 16% of patients SLNB recommendations changed after slide review by expert pathologists[26]. In the current study, the recommendation for SLNB changed in 8.6% after review by an MIA pathologist. In 5.1% of cases, pathology review would have resulted in SLNB being offered to the patient whereas in 3.5% of cases SLNB would not have been offered following MIA pathology review. The change in recommendation for SLNB increased from 7.9% in 2002 to 10.4% in 2010 (Figure 1). This was due to more frequent changes in T-classification in 2010.

Completeness of pathology reports

MIA pathology reports were significantly more complete for the pathologic criteria necessary to define T-classification, select appropriate surgical excision margins, and make SLNB recommendations. Our result demonstrating increased completeness of melanoma pathology highlights the importance of pathology review by expert pathologists.

Table 5. Completeness of histopathology reports for essential pathologic criteria defining T-classification, appropriate surgical excision margins and SLNB recommendations

Pathologic feature	Completeness of pathology reports		Significance
	non-MIA (n=5011)	MIA (n= 5011)	
T-classification	86.6%	97.6%	P<0.001
Surgical excision margins	95.2%	99.6%	P<0.001
SLNB	94.5%	99.4%	P<0.001

SLNB: Sentinel Lymph Node Biopsy. MIA: Melanoma Institute Australia

Factors influencing a change in diagnosis, T-classification or management.

Breslow thickness, type of biopsy and melanoma location were the only factors independently associated with changes in diagnosis, T-classification, recommended surgical excision margins, or SLNB management following MIA review. Melanomas ≤1.00mm were associated with more frequent changes in diagnosis, recommended surgical excision margins, and SLNB management. The association of Breslow thickness and change in diagnosis occurred because the diagnosis often changed in cases of *in situ* melanoma. Due to changes in diagnosis, the recommendations for surgical excision margins and SLNB were also altered. Partially biopsied melanomas (incision, shave, or punch biopsies) were independently associated with more frequent changes in T-classification and surgical/SLNB management when compared to melanomas removed by complete excision biopsy (as recommended in most national melanoma management guidelines). This result reinforces the importance

of complete excision biopsy of the primary melanoma for diagnosis and management. Excision of the entire clinically apparent lesion, with a 1- to 2-mm margin of adjacent normal-appearing skin, is the biopsy technique of choice when melanoma is suspected[6]. Other studies have also shown that partial biopsy leads to more frequent misdiagnosis than excisional biopsy in melanoma[31-37]. Therefore partial biopsies should be avoided whenever possible unless there are sound clinical reasons to deviate from accepted guidelines.

Conclusion

The diagnosis and/or T-classification of melanoma often changed after review of the histopathology by an expert pathologist, often resulting in a change of the recommended clinical management. Partial biopsies were more often associated with a change in diagnosis and/or important pathologic features. Based on our results, we recommend that pathology review be considered not only for all patients attending specialist melanoma treatment centers, but also for melanoma patients being treated elsewhere.

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Chapter 4

The role of microsatellites in staging melanoma

Maarten G. Niebling, Lauren Haydu, Rooshdiya Z. Karim, John F. Thompson, Richard A. Scolyer

In preparation for publication

Abstract

Background

There is no high level of evidence regarding the prognostic relevance of microsatellites (MS). This is partly due to the variety in definition of this pathologic feature in the last three decades. The aim of this study is to determine the prognostic significance of features of MS including presence or absence, count, size and distance from the primary tumor. The outcomes to be investigated include melanoma-specific survival (MSS) and disease-free survival (DFS), as well as the association with sentinel lymph node (SLN) and non-sentinel node (NSN) positivity, and recurrence (local, regional and distant).

Methods

Between 2001 and 2011 87 primary cutaneous melanoma cases were selected from the Melanoma Research Database (MRD) of the Melanoma Institute Australia for which the presence of MS was recorded on the pathology report. Following review of all pathology slides for each tumor, 69 MS cases were retained in the study and manually matched with 69 controls.

Results

The 5-year MSS was worse in the MS group (42.8%) compared to the control group (67.4%) (P=0.032). The 5-year DFS was 18.1% in the MS group and 45.8% in the control group (P<0.001). The distance (mm) of the MS from the primary melanoma was found to significantly influence DFS (HR=1.305, 95% CI: 1.117-1.524, P<0.001) and MSS (HR=1.386 95% CI: 1.101-1.744, P=0.005) in multivariate analysis. Number and size of MS were not significant prognostic factors in this study. The presence of MS was the only factor that proved to be an independent predictor of SLN positivity in the multivariate model (OR 4.636; 95% CI 1.660-12.946; P=0.003), but not NSN positivity. MS were significantly correlated with more loco-regional recurrences (P<0.001) but not distant metastases (P=0.115).

Conclusion

Melanoma with microsatellites is an aggressive tumor, associated with significantly worse MSS and DFS compared with melanoma without microsatellites. The presence of MS is also associated with SN positivity and local and in-transit recurrence. Additionally, the distance the MS has migrated from the primary tumor is an independent prognostic factor that should be routinely recorded by the pathologist.

Introduction

In the current (7th) edition of the American Joint Committee on Cancer staging and classification system, microsatellites (MS) are defined as 'discontinuous nests of metastatic cells more than 0.05mm in diameter that are clearly separated by normal dermis (not fibrosis or inflammation) from the main invasive component of melanoma by a distance of at least 0.3 mm'.[1] The presence of MS in the primary melanoma tumor specimen upstages patients to stage III disease even in cases that lack evidence of regional disease. This categorization is based on studies that show that the presence of MS is associated with an increased frequency of positive sentinel lymph nodes (SLNs) and an increased risk of loco-regional recurrence.[7-10,12-15] Furthermore, several studies have suggested that disease-free survival (DFS) for melanoma patients with MS is similar to DFS for patients with clinically detectable satellite metastases.[4-9] Therefore, presence of this pathologic feature assigns patients to the N2c category of the 7th edition of the AJCC staging system.[1]

MS were first described by Day et al in 1981, who defined MS as a nest of melanoma cells >0.05 mm in diameter, separated from the main body of the tumor mass by a layer of collagen or subcutaneous fat.[4] When comparing the outcome of patients with clinically localized primary cutaneous melanoma, Day et al. found that patients with microsatellites experienced significantly worse 5-year disease-free survival (36%, n=95) compared with patients without microsatellites (89%, n=501) concluding the factor was comparable to ulceration in its prognostic effect.[4]

However, the definition of MS has varied to some degree over the past three decades, and there is no evidence to support the significance of the size of MS or distance of MS from the primary tumor used in the AJCC definition. There is mixed evidence regarding the association of MS with overall survival[4-10] (OS) which could be due to the fact that MS are not frequently observed in primary tumor specimens (4-19%)[4-10], and as a result, most studies are underpowered to observe a difference in OS.

The primary aim of this study was to determine the prognostic value of MS in primary cutaneous melanomas. The secondary aims of the study were to analyze the association of MS with SLN positivity and type of first recurrence, and assess the influence of the number of MS, and the MS' size and distance to the primary tumor on survival for patients with a primary melanoma with MS.

Patients and Methods

Patients with primary invasive cutaneous melanoma, treated at Melanoma Institute Australia (MIA) between 2001 and 2011 were selected from the MIA database for which the presence of MS was recorded (n=87). Parameters extracted from the database included: age, gender, date of primary diagnosis, date and type of recurrence, date of last follow-up, status at last follow-up date, stage (AJCC 7th edition), date and status of therapeutic lymph node dissection (TLND) or sentinel lymph node biopsy (SLNB) and completion lymph node dissection (CLND), primary tumor thickness, ulcerative status, tumor mitotic rate (number of mitoses per mm^2), lymphovascular invasion, regression (early [presence of tumor-infiltrating lymphocytes], intermediate and late), melanoma subtype and predominant cell type.

Histopathologic specimens of the 87 cases were collected and reviewed by a research fellow (M.N.) and melanoma specialist pathologists from the Department of Tissue Pathology and Diagnostic Oncology, Royal Prince Alfred Hospital, Sydney (R.K. and R.S.). After pathologic review, 69 MS cases were eligible for analysis and 18 cases were excluded because the initial diagnosis of MS recorded in the MIA database was not confirmed by M.N., R.K. and R.S. MS were defined as discontinuous nests of melanoma cells more than 0.05mm in diameter, clearly separated by a margin of at least 0.3mm of normal dermis from the main invasive tumor component. For each MS case, the number of MS, size of the largest MS (mm) and greatest distance to the primary tumor (mm) was recorded. These three variables were analyzed as continuous variables as well as categorical variables, with cut-off points based on the median, and 25th and 75th percentiles. The 69 MS cases were matched with 69 control cases without MS for age, gender, year of diagnosis and Tstage (AJCC 7th edition).

Local recurrence was defined as recurrence of melanoma within 5cm of the scar. In-transit recurrence was defined as recurrence occurring outside a 5cm radius from the primary melanoma or scar, but between the primary site and regional node field. For the purposes of analysis, local, in-transit and regional recurrences were grouped together as loco-regional recurrences.

For analysis, the melanoma subtypes were grouped into four categories: (1) superficial spreading melanoma (SSM), (2) nodular melanoma (NM [pure NM and NM with SSM]), (3) desmoplastic melanoma and (4) other (acral lentiginous, lentigo maligna melanoma and blue naevus-like melanoma).

DFS and melanoma-specific survival (MSS) were calculated from the date of primary melanoma diagnosis to the date of first recurrence or death from melanoma, respectively.

IBM SPSS Statistic version 21.0 software (Chicago, IL) was used to perform all statistical analyses. Survival curves were constructed with the Kaplan-Meier method and differences were assessed using the log-rank test. The Cox proportional hazards model (stepwise selection method) was used to perform univariate and multivariate survival analyses and determine hazard ratios (HR) and corresponding 95% confidence intervals (CI). Log minus log plots were visually inspected to confirm no violation of the proportional hazards assumption. Mann-Whitney U, chi-square, and multivariable binary logistic regression methods were used to assess the associations between features of MS, sentinel lymph node (SLN) positivity and non-sentinel node (NSN) positivity.

Results

The study cohort consisted of 69 MS cases and 69 matched control cases. Clinical and histopathologic characteristics of the study population are presented in Table 1. Median follow-up was 18 months (range 6 days to 87 months) in the MS group and 35 months (range 1 to 103 months) in the control group. In the MS group the median number of MS was 1 (range 1 to 10), median size of the MS was 1.20 mm (range 0.09-16.00 mm), and median distance from the primary tumor to the MS was 2.20 mm (range 0.10 to 30.00 mm; Table 2, Figure 1).

Table 1. Clinical and histopathologic features of the overall cohort (n=138)

Factor	Value	MS	Controls	*P*-value
Age	Median (IQR)	66 (58-78)	65 (56-77)	
	Mean (range)	65 (25-92)	65 (29-93)	
Gender	Male	39 (56.5%)	39 (56.5%)	
	Female	30 (43.5%)	30 (43.5%)	
Breslow thickness	Median (IQR)	4.10 (2.30-6.96)	4.10 (2.20-6.00)	
	Mean (range)	5.02 (0.85-16.00)	4.61 (0.40-17.00)	Matched
Ulceration	Absent	43 (62.3%)	43 (62.3%)	Variables
	Present	26 (37.7%)	26 (37.7%)	
Mitotic rate	Median (IQR)	5 (2-10)	5 (2-12)	
	Mean (range)	7 (0-22)	8 (0-34)	
Year of primary diagnosis	Median (IQR)	2007 (2006-2009)	2007 (2006-2008)	
	Mean (range)	2007 (2002-2011)	2007 (2001-2011)	
Follow-up duration	Median (IQR)	17.84 (5.40-31.90)	34.53 (13.19-46.06)	<0.001
(months)	Mean (range)	22.09 (0.20-86.80)	33.70 (1.15-102.80)	
In-transit metastasis	Absent	66 (95.7%)	69 (100.0%)	0.08
	Present	3 (4.3%)	0 (0.0%)	
	SLNB negative	18 (26.1%)	32 (46.4%)	
	SLNB positive / CLND	16 (23.2%)	8 (11.6%)	
Nodal management	SLNB positive / no CLND	7 (10.1%)	2 (2.9%)	0.051
	No SLNB	21 (30.4%)	21 (30.4%)	
	TLND	7 (10.1%)	6 (8.7%)	
Number of positive nodes	Median (IQR)	2 (1-3)	1 (1-3)	0.335
(Stage III/IV)	Mean (range)	3 (1-17)	2 (1-9)	
Nstage (AJCC 7th edition)	N0	0 (0.0%)	53 (76.8%)	
	N1a	0 (0.0%)	7 (10.1%)	
	N1b	0 (0.0%)	3 (4.3%)	
	N2a	0 (0.0%)	3 (4.3%)	<0.001
	N2b	0 (0.0%)	1 (1.4%)	
	N2c	39 (56.5%)	0 (0.0%)	
	N3	30 (43.5%)	2 (2.9%)	
Overall stage (AJCC 7th	I	0 (0.0%)	10 (14.5%)	
edition)	II	0 (0.0%)	43 (62.3%	<0.001
	III	66 (95.7%)	16 (23.2%)	
	IV	3 (4.3%)	0 (0.0%)	

Clark level of invasion	II	1 (1.4%)	0 (0.0%)	
	III	3 (4.3%)	9 (13.0%)	0.091
	IV	37 (53.6%)	41 (59.4%)	
	V	27 (39.1%)	17 (24.6%)	
	Missing	1 (1.4%)	2 (2.9%)	-
Melanoma subtype	SSM	20 (29.0%)	12 (17.4%)	
	SSM with NM	11 (15.9%)	4 (5.8%)	
	NM	21 (30.4%)	32 (46.4%)	
	Acral lentiginous	2 (2.9%)	1 (1.4%)	0.020*
	Desmoplastic	6 (8.7%)	17 (24.6%)	
	Lentigo maligna melanoma	2 (2.9%)	2 (2.9%)	
	Malignant blue naevus	2 (2.9%)	0 (0.0%)	
	Missing	5 (7.2%)	1 (1.4%)	-
Predominant cell type	Spindle	7 (10.1%)	19 (27.5%)	
	Epithelioid	50 (72.5%)	29 (42.0%)	<0.001
	Mixed	10 (14.5%)	21 (30.4%)	
	Missing	2 (2.9%)	0 (0.0%)	-
Regression	Absent	29 (42.0%)	19 (27.5%)	
	Early (TILs)	32 (46.4%)	43 (62.3%)	0.138
	Intermediate	1 (1.4%)	3 (4.3%)	
	Late	1 (1.4%)	3 (4.3%)	
	Missing	6 (8.7%)	1 (1.4%)	-
Neurotropism	Absent	67 (97.1%)	64 (92.8%)	0.245
	Present	2 (2.9%)	5 (7.2%)	
Lymphovascular invasion	Absent	49 (71.0%)	65 (94.2%)	<0.001
	Present	20 (29.0%)	4 (5.8%)	
Associated naevus	Absent	55 (79.7%)	50 (72.5%)	
	Dysplastic	4 (5.8%)	14 (20.3%)	
	Dermal	4 (5.8%)	2 (2.9%)	0.053
	Congenital	1 (1.4%)	0 (0.0%)	
	Compound	0 (0.0%)	2 (2.9%)	
	Missing	5 (7.2%)	1 (1.4%)	-

Factors Associated with MS

The presence of lymphovascular invasion was significantly associated with the presence of MS (*P*<0.001). Furthermore, epithelioid cell type was observed in 72.5% of MS cases compared with 42.0% of control cases (*P*<0.001). In addition, SSM was associated with the presence of MS and desmoplastic melanomas were more frequently observed in the control group (*P*=0.020). Although not significant, the MS group had a lower proportion of associated dysplastic naevi compared with the control group (5.8% and 20.3%; *P*=0.053).

Table 2. Features of microsatellitosis

CONTINUOUS MEASURES		
Feature	Mean (range)	Median (IQR)
Microsatellites (number)	2 (1-10)	1 (1-3)
Microsatellite size	1.94 (0.09-16.00)	1.20 (0.60-2.05)
Microsatellite distance to primary	3.25 (0.10-30.00)	2.20 (1.00-3.75)
CATEGORIES		
Feature	Categories	N (%)
Microsatellites (number)	N=1	41 (59.4%)
	N>1	28 (40.6%)
	0.01-0.60 mm	19 (27.5%)
Microsatellite size	0.61-1.20 mm	16 (23.2%)
	1.21-2.05 mm	17 (24.6%)
	>2.05 mm	17 (24.6%)
	0.01-1.00 mm	18 (26.1%)
Microsatellite distance to primary	1.01-2.20 mm	17 (24.6%)
	2.21-3.75 mm	17 (24.6%)
	>3.75 mm	17 (24.6%)

Recurrence and Survival in the Overall Cohort

A total of 56 patients in the overall cohort experienced a recurrence during the follow-up period. In the MS group 50.7% experienced a recurrence compared with 30.4% in the control group (*P*=0.015). Specifically, 49% and 29% of MS cases experienced a loco-regional or distant recurrence respectively, compared with 20% and 19% of cases without MS (P<0.001 and P=0.115). The MS group had a significantly worse disease-free survival with 27.1% and 18.1% cumulative DFS at 3 and 5 years, respectively, compared with 70.0% and 45.8% in the control group (*P*<0.001) (Figure 2). This difference was still significant after adjustment for other variables in the multivariate model (HR 3.33; 95%CI 1.83-6.06; *P*<0.001; Table 3). Three and 5-year cumulative MSS was 65.2% and 42.8% in the MS group and 86.1% and 67.4% in the control group, respectively (*P*=0.032) (Figure 2). This difference was also observed on multivariate analysis (HR 2.47; 95% CI 1.12-5.41; *P*=0.024; Table 3).

Table 3. Disease-free and melanoma-specific survival in the overall cohort

Factor	Value	Disease-free Survival n=132		Melanoma-specific Survival n=136	
		Hazard Ratio (95% CI)	***P*-value**	**Hazard Ratio (95% CI)**	***P*-value**
Multivariate Model 1					
Age	Years	1.021 (0.995-1.049)	0.116	1.000 (0.969-1.033)	0.979
Gender	Male	0.986 (0.552-1.764)	0.963	0.436 (0.173-1.095)	0.077
Thickness	Mm	1.059 (0.961-1.167)	0.245	1.163 (1.050-1.289)	0.004
Ulceration	Present	0.897 (0.472-1.706)	0.741	1.286 (0.511-3.240)	0.593
Mitotic rate	Per mm^2	1.043 (0.997-1.091)	0.065	1.081 (1.019-1.148)	0.010
SLNB	Conducted	0.772 (0.396-1.507)	0.449	0.615 (0.251-1.506)	0.287
Positive nodes	Number	1.261 (1.112-1.431)	<0.001	1.121 (0.978-1.285)	0.100
Microsatellites	Present	3.334 (1.834-6.060)	<0.001	2.466 (1.124-5.409)	0.024
*Multivariate Model 2**					
Microsatellites	Number	1.144 (1.013-1.293)	0.031	1.219 (1.013-1.467)	0.036

Abbreviations: CI = confidence interval; SLNB = sentinel lymph node biopsy

*Adjusting for all variables in Model 1 (Age, Gender, Thickness, Ulceration, Mitotic Rate, SLNB, Positive Nodes)

Sentinel and Non-sentinel Lymph Node Positvity

SLN biopsy was conducted in 41 MS patients and 42 controls. On univariate analysis, the presence of MS (*P*=0.003), lymphovascular invasion (*P*=0.039) and epithelioid cell type (*P*=0.027) were associated with a positive SLN biopsy result. The presence of MS was the only factor that proved to be an independent predictor of SLN positivity after adjustment for other variables in the multivariate model (OR 4.64; 95% CI 1.66-12.95; *P*=0.003). No significant predictors of NSN positivity were identified on either univariate or multivariate analysis (n=24).

Prognostic Features of MS

A sub-group analysis of the 69 MS cases was performed to determine the influence of the distance to the primary tumor, the size and number of MS on DFS and MSS. The number of MS did not influence DFS nor MSS on univariate and multivariate analysis (*P*=0.691 and *P*=0.865, respectively; Table 4).

The distance of the MS to the primary melanoma as a continuous variable was found to significantly influence DFS and MSS on univariate analysis (*P*<0.001 for both). These differences retained significance in the multivariate model (HR 1.31; 95% CI 1.12-1.52; *P*<0.001 and HR 1.39; 95% CI 1.10-1.74; *P*=0.005, respectively). When distance to the primary tumor was analyzed as a categorical variable, MS distance 2.21-3.75 mm and >3.75 mm had a significantly worse prognosis compared with MS distance 0.01-1.00 mm, in terms of DFS (*P*=0.045 and *P*=0.013, respectively; Table 3). Moreover, distance categories 1.01-2.20mm, 2.21-3.75mm and >3.75mm were all associated with reduced MSS compared

with MS distance 0.01-1.00 mm (*P*=0.024, *P*=0.045 and *P*=0.014, respectively; Table 4).

The MS size as a continuous variable significantly influenced MSS on univariate analysis (*P*=0.018). However, this difference lost significance on multivariate analysis (*P*=0.234). On univariate and multivariate analysis, the size of the MS as a continuous variable did not influence DFS (*P*=0.263 and *P*=0.535, respectively). When the MS group was divided into two categories, based on the MS size with a cut-off point at 1.20 mm, the >1.20 mm group had significantly reduced DFS on univariate analysis (*P*=0.007). Although, this significant difference was lost in the multivariate model (*P*=0.085; Table 4). No significant differences in MSS were observed between the two categories on both univariate (*P*=0.834) and multivariate analysis (*P*=0.075; Table 4).

Discussion

The present study confirms that the presence of MS in melanoma patients negatively influences MSS and DFS, and increases the likelihood of sentinel node positivity and loco-regional recurrence. Additionally, the current findings indicate that an increased distance of the microsatellite from the primary tumor conveys a worse prognosis both in terms of MSS and DFS. However, the size or number of microsatellites present in the primary tumor specimen was not prognostic in this cohort.

Several studies have shown that the presence of MS is a predictor of reduced overall and disease-free survival.[4,6-10] The 5-year cumulative overall survival (OS) ranged from 34% to 40% in the majority of these studies, and 5-year cumulative DFS ranged from 18% to 36%.[4,6,8,10] These results are comparable with the results of the present study of 42% and 18% for MSS and DFS, respectively, for the MS group and were confirmed in multivariate analysis.

Of the previous studies only Leon et al conducted a case-control study.[6] Moreover, different definitions were used in various studies to describe MS. Nagore et al showed OS and DFS for MS cases of 70.4% and 51.8%, respectively, although survival was still significantly worse for MS cases compared with the cohort of patients without MS. However, this study had a melanoma patient cohort with non-aggressive pathologic features, as median Breslow thickness was only 1.2mm and ulceration was present in only 21% of the melanomas. In comparison, other studies have shown that melanomas with MS were associated with aggressive pathologic features. For example, in a study conducted by Kimsey et al, median Breslow thickness was 5.4mm and 71% of melanomas showed presence of ulceration.[10] In our study, median Breslow thickness was 4.1mm and 62% of the melanomas were ulcerated. This also shows the importance of matching MS cases and controls on Breslow thickness and ulceration.

Our study results showed that presence of MS was also associated with lymphovascular invasion. Moreover, lymphovascular invasion showed to be a prognostic factor for worse MSS and DFS in the univariate analysis and lymphovascular invasion was also correlated with SLN positivity. Lymphovascular invasion has shown to be an important prognostic factor in certain cancer, such as breast cancer.[16] In cutaneous melanoma prognostic significance of lymphovascular invasion remains unclear as several studies failed to show to show any prognostic value of this pathologic feature.[17-22] However, several

other studies, including the present study in the univariate analysis, showed lymphovascular invasion to be a prognostic factor for poor survival and presence SLN positivity.[9,23-29] The association of lymphovascular invasion and MS could be explained by the theory that MS is the result of lymphovascular transport of tumor cells in the vicinity of the primary tumor. Moreover, it seems logical that the further away MS are transported the higher the impact is of MS on MSS and DFS. This theory is supported by our study results, as increasing distance between MS and the primary tumor was associated with

Table 4. Disease-free and melanoma-specific survival in the microsatellite sub-group

Variable	**Value**	**Disease-free Survival n=67**		**Melanoma-specific Survival n=67**	
		Hazard Ratio (95% CI)	***P*-value**	**Hazard Ratio (95% CI)**	***P*-value**
Multivariate Model 1					
Age	Years	0.991 (0.956-1.029)	0.647	1.021 (0.965-1.080)	0.478
Gender	Male	1.559 (0.699-3.477)	0.278	2.687 (0.689-10.471)	0.154
Thickness	Mm	1.188 (1.024-1.378)	0.023	1.211 (1.011-1.450)	0.037
Ulceration	Present	0.471 (0.169-1.317)	0.151	0.532 (0.105-2.708)	0.448
Mitotic rate	Per mm²	1.065 (0.985-1.151)	0.112	1.118 (0.996-1.255)	0.059
SLNB	Conducted	0.838 (0.325-2.161)	0.715	2.040 (0.440-9.451)	0.362
Positive nodes	Number	0.974 (0.802-1.182)	0.786	1.095 (0.898-1.335)	0.369
Microsatellites	# (continuous)	0.820 (0.656-1.025)	0.082	0.758 (0.528-1.089)	0.134
Microsatellites size	mm (continuous)	1.082 (0.844-1.387)	0.535	0.783 (0.523-1.172)	0.234
Microsatellites distance	mm (continuous)	1.305 (1.117-1.524)	<0.001	1.386 (1.101-1.744)	0.005
Multivariate Model 2*					
Microsatellites	N>1	1.001 (0.438-2.290)	0.998	1.152 (0.345-3.842)	0.818
Microsatellite size	>1.20mm	2.048 (0.906-4.627)	0.085	0.333 (0.099-1.118)	0.075
Microsatellites distance	>2.20mm	2.031 (0.874-4.716)	0.099	4.124 (0.843-20.171)	0.08
Multivariate Model 3*					
Microsatellites	N>1	1.059 (0.430-2.608)	0.901	2.712 (0.592-12.430)	0.199
Microsatellite size	0.01-0.60 mm	Reference			
	0.61-1.20 mm	0.946 (0.318-2.818)	0.921	1.449 (0.272-7.727)	0.664
	1.21-2.05 mm	2.411 (0.785-7.407)	0.124	0.161 (0.018-1.485)	0.107
	>2.05 mm	0.967 (0.252-3.705)	0.961	0.273 (0.036-2.066)	0.209
Microsatellite distance	0.01-1.00 mm	Reference			
	1.01-2.20 mm	3.231 (0.954-10.942)	0.06	18.928 (1.485-241.280)	0.024
	2.21-3.75 mm	3.923 (1.033-14.892)	0.045	13.220 (1.061-164.690)	0.045
	>3.75 mm	5.569 (1.427-21.737)	0.013	26.944 (1.935-375.207)	0.014

Abbreviations: MS = microsatellites; CI = confidence interval; SLNB = sentinel lymph node biopsy.

*Adjusting for all variables in Model 1 (Age, Gender, Thickness, Ulceration, Mitotic Rate, SLNB, Positive Nodes)

reduced MSS and DFS. However, the size and number of MS did not influence prognosis.

For DFS, apart from MS, positive nodes showed to be an independent prognostic factor, which was already concluded previously.[1] Recurrences occurred in 50.7% of patients in the MS group, compared with 30.4% in the control group (*P*=0.015). This is in concordance with other studies that analyzed recurrences in MS cases.[7,8,10,15] The majority of first recurrences in MS cases was local or in-transit. This observation could be explained by the hypothesis that these local recurrences are actually not a recurrence, but were MS that were already spread so far from the primary tumor that they were not excised with wide excision of the primary tumor. Two other studies also showed association between MS and local recurrence.[7,15] Shaikh et al also showed an association of MS with regional recurrences.[8] Although the present study did not show the association of MS with regional recurrences, there was an association with SLN positivity. The presence of MS was the only prognostic factor that independently predicted SLN positivity, several other studies also showed MS to be a prognostic factor for SLN positivity.[5,10,12-14] Presumably, the development of MS involves a continuum from MS to in-transit and regional node field metastases. This idea is supported by the present study, as the presence of MS is significantly associated with in-transit recurrence as a first event and presence of positive SLNs. Figure 1 shows that all MS cases in the present study were in concordance with the latest definition formed by the AJCC (any discontinuous nest of metastatic cells more than 0.05mm in diameter that are clearly separated by normal dermis (not fibrosis or inflammation) from the main invasive component of melanoma by a distance of at least 0.3 mm). However, we think that the definition should not be emphasized on the size or distance of MS to the primary tumor. There is no evidence for the

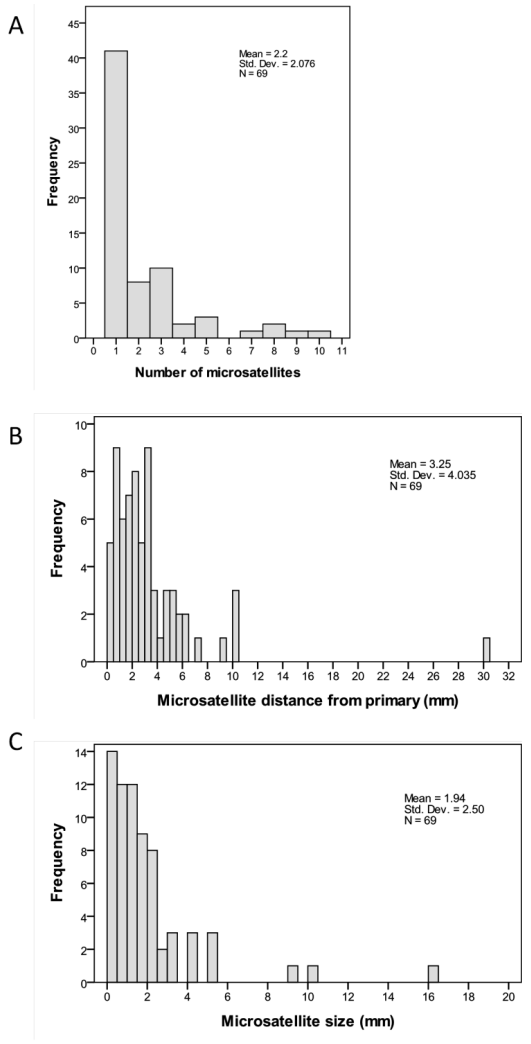

Figure 1. (A) Number, (B) size and (C) distance from primary tumor for all microsatellites

specific value of the MS' size or distance to primary tumor in the AJCC definition of MS. We think that the emphasis of the definition should be on the clear separation of primary tumor and MS by normal dermis. Diagnosis of MS is associated with a high rate of false positives. This is due to the fact that the presence of an isolated nest of tumor cells adjacent to the primary tumor can represent an extension of the primary tumor that has been cut tangentially or in cross-section. Preferably, serial sectioning should be performed on the tissue blocks to prevent a false positive diagnosis of MS.

Our results support a theory for a pathway in the initial metastatic cascade of cutaneous melanoma. MS could be due to the lymphovascular transport of tumor cells from the primary tumor, followed by further spread to in-transit metastasis and/or spread to regional lymph nodes. More studies on the prognostic and biological significance of MS are needed in larger, potentially multi-institutional cohorts to overcome the challenge of a rarely observed feature, 4%-19% in the literature.[4-10]

Moreover, in the MIA database, the presence or absence of MS was reported in only 2.9% of 4313 pathology reports by MIA-affiliated pathologists. The current study points to not only the importance of recording the presence or absence of microsatellites, but also the greatest distance of the microsatellites from the primary tumor. In summary, presence of MS is associated with the presence of positive SLNs, increased likelihood of loco-regional recurrences and subsequently reduced DFS and MSS. There is no doubt MS will continue to be a factor that influences the staging and clinical management of melanoma patients.

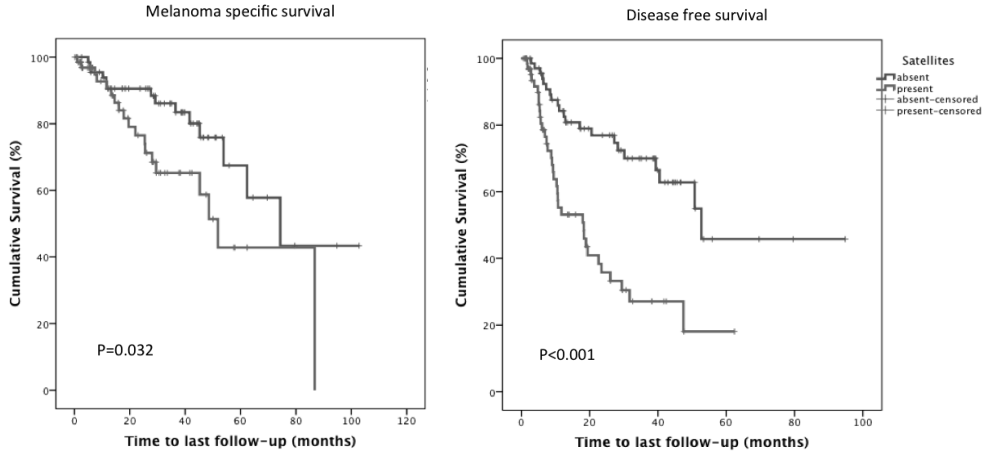

Figure 2. (A) Melanoma specific survival and (B) Disease free survival for microsatellite cases and control cases

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Part II

Management of stage III melanoma patients

Chapter 5

A systematic review and meta-analyses of sentinel lymph node identification in breast cancer and melanoma, a plea for tracer mapping

M.G. Niebling, R.G. Pleijhuis, E. Bastiaannet, A.H. Brouwers, G.M. van Dam, H.J. Hoekstra

Submitted

Abstract

Purpose

Sentinel lymph node biopsy (SLNB) has become a widely accepted staging procedure for both breast carcinoma and melanoma. The aim of our study was to systematically review different SLNB techniques and perform a meta-analysis for corresponding identification and false-negative rates.

Methods

A systematic review of the literature on SLNB in patients with early-stage breast carcinoma and melanoma was performed. Only original study groups were included. The SLN identification rate and false negative rate were pooled for patients with breast carcinoma or melanoma according to radiocolloid tracer, blue dye, indocyanine green (ICG), or a combination of a radiocolloid tracer with a blue or ICG.

Results

Between 1992 and 2012, a total of 154 studies (88 breast carcinoma and 66 melanoma) were reported that met our eligibility criteria. These studies included a total of 44,172 patients. The pooled SLN identification rate in breast carcinoma and melanoma patients using solely blue dye was 85% (range: 65-100%) and 84% (range: 59-100%), while for radiocolloid alone it was 94% (range: 67-100%) and 99% (range: 83-100%), respectively. Using a combination of radiocolloid and ICG, identification rates of 96% (range 94.9-96.7%) and 100% (range: 100-100%) were reported.

Conclusions

The current meta-analysis provides data that favors the use of radiocolloid or radiocolloid combined with a blue dye for the identification of the SLN. Performing SLNB with radiocolloid alone is the technique of choice for experienced surgeons, since blue dye has multiple disadvantages. SLNB using ICG as a fluorescent dye seems a promising technique for the near future.

Introduction

Breast carcinoma and melanoma are annually diagnosed in 1.38 million and 197,000 people, representing 10.9% and 1.6% of all cancers in the world, respectively.[1] Fortunately, the majority of both cancers are initially diagnosed stage I or II.[2]

The lymphatic route is a principal way for breast carcinoma and melanoma to metastasize from their original focus. Cancer cells progressing via the lymphatic vessels are trapped in the first lymph node they encounter, denominated as the Sentinel Lymph Node (SLN).[3,4]

Lymph node metastases can either be detected clinically or through the use of a Sentinel Lymph Node Biopsy (SLNB). The concept of the SLN was first described in 1960 by Gould et al and is based on two basic principles: the existence of an orderly and predictable pattern of lymphatic drainage to a regional lymph node basin, and the functioning of a first lymph node as an effective filter for tumor cells.[5,6] Clinical implementation of the concept was deployed on a broad scale by Cabanas et al in penile cancer.[7]

Morton et al described a method for SLNB in 1992, using peritumoral intradermal injections of blue dye in patients with primary cutaneous melanoma.[4] The blue-stained lymphatic vessel was followed surgically until it was seen entering a blue-stained lymph node. In 1993, Alex et al added the use of a radiotracer, injected intradermally around the primary tumor site, followed by imaging and subsequent intraoperative use of a handheld gamma probe to localize and extirpate the SLN.[8] In the same year, Krag et al described the application of this technique in breast carcinoma patients.[9] Albertini et al combined both the blue dye and the radioactive tracer in 1996, which is currently the most commonly used technique for SLNB in most centers.[10] The two most widely accepted clinical applications of SLNB are for cutaneous melanoma ≥1mm and T1-2 breast carcinoma.[11-13] SLNB is preferred over direct lymph node dissection (LND) because the risk of morbidity is lower.[14,15] Moreover, SLNB allows the pathologist to study the few removed SLNs in greater detail for tumor burden compared with examination of the large number of lymph nodes removed by LND.[16]

Despite its wide global use, the SLNB procedure has not been standardized internationally due to variation in the method and material used between surgeons and institutions. Although a combination of blue dye and a radiotracer is considered standard-of-care, a significant number of surgeons work with blue dye or a radiotracer alone. In addition, the fluorescent optical contrast agent indocyanine green (ICG) was recently introduced in the clinic as an alternative agent for SLNB guidance.[17]

In the present study, a systematic review was performed on peer-reviewed scientific articles to evaluate different SLNB techniques and their corresponding identification- and false-negative rates in breast carcinoma and melanoma. In addition, we aimed to identify technical aspects influencing the outcome of the SLNB procedure.

Methods

Literature search, inclusion and exclusion criteria

A comprehensive, systematic review was conducted in October 2012 of the medical literature published after 1992. Details of the methods of the search and inclusion/exclusion criteria are specified in Supplementary file 1.

Study quality assessment

Authors MGN and RGP performed the quality assessment of selected articles. Study quality was evaluated using the "QUADAS" (Quality Assessment of Diagnostic Accuracy Studies) tool to extract relevant study design characteristics.[18] The core QUADAS items used in this review are outlined in Supplementary file 2. In brief, QUADAS items included: availability of representative spectrum of patients, clear description of selection criteria, adequate reference standard, execution of index and reference test described in sufficient detail, independent interpretation of index test, availability of relevant clinical data, reporting of uninterpretable results, adequate explanation of study withdrawals, availability of adequate reference standard for verification purposes, adequate follow-up, and reporting of false negatives. Each item was scored as 'yes', 'no', or 'unclear'.

Statistical analyses

Univariate and multivariable logistic regression analyses were performed to identify factors associated with SLN identification and false-negative rates for the different SLNB techniques. Analyses were stratified to technique and performed separately for breast carcinoma and melanoma. Factors included in this analysis were: type of study (prospective or retrospective), study period (1992-2000, 2001-2006, or 2007-2012), age (in years), definition of SLN (radioactivity higher than background or 10% rule), tumor size (in mm), number of SLNs removed, quality of the study (QUADAS score), type of dye (patent blue, isosulfan blue, lymphozuran blue, methylene blue, a combination of blue dyes, ICG, or unknown) or radiocolloid (^{99m}Tc-sulfer colloid, ^{99m}Tc-tin colloid, ^{99m}Tc-phytate, ^{99m}Tc-human serum albumin, ^{99m}Tc-dextran 500, or ^{99m}Tc-tilmanocept), concentration of dye or radiocolloid (in mg/ml or in Mbq), injected volume of dye or radiocolloid (in ml), injection site (peritumoral, parenchymal, subareolar, intradermal, subdermal, combination, or unknown), timing of injection (<15 minutes, 15 minutes-1 hour, 1-10 hours, >10 hours), and massage after injection of dye or radiocolloid.

The false negative rate (FNR) was defined as the probability of a negative SLN when the patient has positive lymph nodes in the LND or in the follow-up period of the study.

Calculations were performed using the statistical packages SPSS (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY:). P-values <0.05 were considered statistically significant.

Meta-analysis

Identification rate (IR) and FNR were extracted from the studies and pooled (on a study level) ac-

cording to SLNB technique, breast carcinoma or melanoma, and period (1992-2000, 2001-2006 and 2007-2012). Summary identification- and false-negative rates and the corresponding 95% Confidence Interval (CI) were calculated using STATA/SE version 12.0 (StataCorp, College Station, Texas, USA) using the metan command. Pooled values were calculated, using either a random-effects or a fixed-effects model, depending on the number of included studies and the amount of heterogeneity observed.

Results

Literature search

Our comprehensive literature search resulted in 335 studies that were selected for further analysis, dating from January 1992 to October 2012. Of these studies, 198 comprised SLNB in breast carcinoma and 137 in melanoma. After thorough review, 154 studies (88 breast carcinoma and 66 melanoma) met our inclusion criteria (Tables 1-3). A total of 108 out of 154 studies reported on the IR of SLNB using blue dye only, 111 on using radiocolloid only, and 118 on using the combination of blue dye and radiocolloid. Nineteen studies analyzed the IR of SLNB using ICG solely and 12 studies analyzed ICG in combination with radiocolloid. When data from all included articles is aggregated, a total of 44,172 patients underwent SLNB with a median of 103 patients (range: 4-5,611) per study. In the studies where the number of SLNs identified was reported, blue dye identified 12,623 SLNs in 6,052 patients with a mean of 2.09 SLNs per patient, radiocolloid identified 1,6175 SLNs in 7,455 patients with a mean of 2.17 SLNs per patient, blue dye and radiocolloid both identified 37,680 SLNs in 19,917 patients with a mean of 1.89 SLNs per patient. The SLN was positive in 14.6% in SLNs detected with blue dye, 11.5% detected with radiocolloid, and 23.9% detected with both blue dye and radiocolloid. ICG identified 2,697 SLNs in 947 patients with a mean of 2.85 SLNs per patient. Combined with radiocolloid, ICG identified 353 SLNs in 194 patients with a mean of 2.87 SLNs per patient. Table 1 and Table 2 provide an overview of the number of patients per study, the mean SLN, IR, FNR and the duration of follow-up for all breast carcinoma and melanoma studies, respectively. In Table 3, an overview is shown for SLNB using ICG alone or combined with radiocolloid.

Identification rate and false negative rate

IR and FNR for SLNB using solely blue dye, solely radiocolloid, and these two combined are presented in Table 1 and Table 2, for breast cancer and melanoma patients, respectively. IR and FNR for SLNB for SLNB using ICG, either solely or combined with radiocolloid and/or blue dye, are presented in Table 3. The pooled IR and FNR for all techniques are presented in Tables 4 and 5, pooled per tumor type for the time periods 1992-2000, 2001-2006, and 2007-2012.

Multivariate analysis

In breast carcinoma, multivariate factors significantly associated with SLN IR were the following: when solely blue dye was used in SLNB (median: 0.87), massage of the breast following injection of

blue dye (vs. no massage; OR 3.5, range: 1.2-10.6, *P*=0.02) was identified as an independent factor to increase SLN detection. When solely radiocolloid was used (median: 0.96), a prospective study design (vs. retrospective; OR 6.9, range: 1.3-38.4, *P*=0.03) was associated with a higher SLN detection rate. When radiocolloid and blue dye were combined (median: 0.97), SLN detection rate was found to increase with ascending year of publication since the time period of 1992-2000 (vs. 2001-2006; OR 13.0, range: 1.4-119.1 and 2007-2012; OR 20.0, range: 2.0-203.3, *P*=0.04). No independent factors were identified for SLN detection rate when solely ICG (median: 0.98) or a combination of radiocolloid and ICG (median: 0.96) was used.

In melanoma, multivariate factors significantly associated with SLN IR were the following: when solely ICG was used, the volume of injection (in ml) was associated with SLN IR (OR 9.5, range: 1.1-71.0, *P*=0.03). When radiocolloid and blue dye were combined, the volume of injection of blue dye was identified as an independent factor (OR 19.7, range: 1.3-289.1, *P*=0.04). No significant factors were identified for the use of solely blue dye, radiocolloid, and radiocolloid combined with ICG.

In addition to SLN IRs, logistic regression was performed to identify factors associated with FNRs. Again, analyses were stratified to technique and performed separately for breast carcinoma and melanoma. In both breast carcinoma and melanoma, no univariate or multivariate significant predictive factors were identified for any of the techniques.

Discussion

SLNB is a widely accepted prognostic procedure in breast carcinoma and melanoma patients. Although a survival benefit compared to watchful waiting with ultrasound is yet to be shown, the status of the SLN is the most important predictor of prognosis and tumor recurrence for early breast carcinoma and melanoma.[19-21] In contrast to the worldwide acceptation of the procedure, controversies remain regarding the technical aspects of the procedure. The current study represents a systematic review of the published literature on SLNB in breast carcinoma and melanoma patients to evaluate the different SLNB techniques and judge them on their merits.

Identification rates of SLNB using blue dye and radiocolloid

The present study showed that pooled SLN IRs in breast carcinoma/melanoma patients are high for SLNB using solely blue dye (85/84%), solely radiocolloid (94/99%), and blue dye and radiocolloid combined (95/98%) and that FNRs are low (3.2/6.1%, 2.2/3.4%, 1.5/2.6%, respectively). In addition, IRs for all studied techniques steadily increased over time. For SLNB using solely radiocolloid, the IR of SLNB in breast carcinoma/melanoma patients has increased from 88/100% in 1992-2000 to 97/100% in 2007-2012. In the latter period, IR was similar compared to SLNB using a combination of radiocolloid with blue dye (97/99%). The increase in IR during the last 18 years is likely due to the increase in gained experience by the surgeons performing SLNB. Several studies have already reported that with the increase of gained experience by the surgeons, the IR of SLNB with solely radiocolloid also steadily

increased with a decline in the marginal benefit offered by using blue dye.[22,23]

In addition, there are some disadvantages of the use of blue dye. First, the use of blue dye as a guide in SLNB may lead to increasing tissue damage when tracing blue-stained lymphatics to the SLN compared to the use of a gamma probe to guide the path of dissection.[24] Second, allergic reactions to blue dye are seen in 0.14-3% of the patients, including urticaria, skin rash, erythema, blue hives, cardiovascular collapse, and anaphylactic shock.[25-31] Other side effects are temporary skin tattooing, blue discoloration of the operative field following peritumoral injection, blue-colored urine for up to 24 hr following administration, and a factitious drop in intraoperative oxygen saturation measured by pulse oximetry.[24] Furthermore, pregnancy is a relative contraindication due to the unknown teratogenicity and long-term toxicity to the fetus.

However, this should not preclude the use of blue dye by those surgeons who have mastered the technique of blue dye and have produced reliable, high IRs and low FNRs. SLN mapping with a radiotracer is expensive and availability is limited to large hospitals, therefore it is not available in most developing countries.[32] Hence, SLN mapping using blue dye solely should be encouraged in hospitals where radiotracers are not available, since the IR for using solely blue dye is acceptable.

Nevertheless, in the current study, the SLN IR using solely blue dye was found to be 8% lower compared to using solely radiocolloid and radiocolloid combined with blue dye. Furthermore, the results of the current study indicate that, in the present era, the addition of blue dye to radiotracers does not increase the SLN IR. Taken together with the disadvantages of blue dye, we advise surgeons, working in hospitals where radiotracers are available and experienced with the radiocolloid SLNB technique, to perform SLNB using solely radiocolloid.

False negative rate

The pooled FNR for all techniques in all articles was at its peak in the last period (2007-2012), while the IR of SLNB has increased over time. This apparent contradiction might be due to the fact that only few surgeons were experienced with the SLNB procedure shortly after its introduction. When the procedure got widely accepted, less experienced surgeons had to perform SLNB, leading to an increased FNR. It is expected that the FNR will decline when surgeons are more familiar with the SLNB technique.

Type of dye

Apart from the debate on whether SLNB should be performed with blue dye, radiocolloid or a combination of the two, there are also controversies on the type of dye or radiocolloid. For both breast carcinoma and melanoma studies included in this review, we analyzed different types of blue dye (Patent blue, Isosulfan blue, Lymphozuran blue, or Methylene blue) or radiocolloid (^{99m}Tc-sulfer colloid, ^{99m}Tc-human serum albumin, ^{99m}Tc-dextran 500, ^{99m}Tc-phytate, and ^{99m}Tc-Tilmanocept). In the present study, univariate or multivariable analysis did not show an association with either the type of blue dye or the type of radiocolloid on IR or FNR. According to these results, the choice of the type of dye/radio-

colloid should be based on the surgeon's preference or on the type of dye with the least side effects.

Injection site

Another issue in lymphatic mapping concerns the optimal injection site for dye/radiocolloid in patients with breast carcinoma. Whereas a consensus exists on injection sites for SLNB in melanoma patients (intradermal injection),[24] this is currently not the case in breast carcinoma.

In the studies included in this review, blue dye and radiocolloid were injected intradermally (18 and 19 studies), subdermally (4 and 4 studies), intraparenchymally (8 and 3 studies), and subareolarly (12 and 12 studies). The present study showed no difference in IR for different injection sites. The choice on injection site can therefore be based on the surgeon's preference, taking previously reported practical advantages of the different injection sites into consideration.[33-36] The injection of blue dye and radiocolloid in breast carcinoma is presented in Figure 1.

Injection volume and massage

In breast carcinoma, a larger volume of injected blue dye was associated with a higher SLN IR, although this was only the case when blue dye was combined with radiocolloid. It is unclear why the volume of injected dye is only associated with a higher IR when using the combined technique. When SLNB was performed using solely blue dye, massage of the breast after dye injection was associated with higher IRs. Postinjection massage increases the flow of lymph fluid, significantly increasing the entry of blue dye and radiocolloid into the lymphatic capillaries.[24]

Lymphoscintigraphy

Lymphoscintigraphy was not used in 4 of 126 studies (3.2%, all breast carcinoma patients) that used radiocolloid solely or in combination with blue.[37-40] In these studies, only a hand-held gamma probe was used before incision to identify hot spots representing the location of the SLNs. Preoperative lymphoscintigraphy is important for providing a road map to guide the surgeon in identifying the regional nodal basin and estimating the location of SLNs.[41] It is used to identify the lymph drainage basin, determine the number of SLNs, differentiate SLNs from subsequent nodes, locate the SLN in an unexpected location, and mark the SLN over the skin for biopsy. Moreover, single-photon emission computed tomography/computed tomography (SPECT/CT) has shown important benefits, as a complementary modality for planar lymphoscintigraphy, in sentinel lymph node mapping.[42] This type of image fusion provides better anatomical benchmarks, provides schematic information about the sentinel node site, and (perhaps most importantly) is easy to understand for surgeons, medical staff, and patients.[43] Lymphoscintigraphy and SPECT/CT images of a melanoma patient are presented in Figure 2.

Definition of sentinel lymph node

Despite ongoing discussions, there is no consensus on the clinical definition of a SLN.[24]

For this reason, a fair number of studies that used unconventional definitions were excluded from our study.[10,46-53] In the present review only studies were included that defined a hot node as 'nodes hotter than the background' or as 'nodes with more than 10% of the hottest node's radioactivity' (Supplementary File 1). The use of either the first or the latter definition did not influence IRs or FNRs for SLNB in both breast carcinoma and melanoma patients, suggesting that less lymph nodes were removed when using the 10% rule while the IR and FNR remained similar.

Near-infrared fluorescence imaging with indocyanine green

As an alternative to the conventional SLNB procedure, SLNB using the fluorescent dye ICG was recently introduced in the clinic.[17] ICG has been used for the last three decades in patients for the study of organ perfusion and ophthalmology and has a safe and well–known pharmacological profile. When excited at the appropriate wavelength, ICG emits photons in the near-infrared fluorescence (NIRF) range of around 800 nm.

Because near-infrared light is invisible to the human eye, a special optical imaging system is needed to visualize the near-infrared signal in the surgical field. Already, several studies have reported on a prototype NIRF optical imaging system, with which the SLN can be detected non-invasively with high accuracy and sensitivity following subcutaneous injection with ICG.[17,32,44-46] After injection, ICG flows along with the lymph fluid and accumulates in the SLNs within minutes, enabling rapid detection and visualization of SLNs as fluorescent hot spots (Figure 3). No adverse reactions were reported in any of the conducted trials.

NIRF optical imaging enhanced with ICG offers some considerable advantages to the current SLN procedure: the technique offers a high resolution, is relatively cheap, makes use of non-ionizing radiation and offers high sensitivity and specificity rates.[47] The most important limitation of fluorescence imaging is the limited penetration depth of optical signals due to the absorption and scattering of photons when propagating through tissue.[48] The use of fluorescent dyes in the near-infrared spectral range, e.g. ICG, reduces absorption and scattering of photons true tissue, thereby increasing tissue penetration of the optical signal up to several centimetres. Furthermore, tissue autofluorescence in the near-infrared range is minimized with an increased signal-to-noise ratio.[48]

In the present study, the mean number of identified SLNs per patient when using ICG was considerably higher (ICG: 2.85 SLNs, ICG+radiocolloid: 2.87 SLNs) compared to solely blue dye (2.09 SLNs), solely radiocolloid (2.17 SLNs) or a combination of the two (1.89 SLNs). This difference might be explained by the small molecular size of ICG, facilitating rapid diffusion throughout the lymphatic system. Indeed, IRs up to 100% are described only 5 to 15 minutes following peritumoral injection when using solely ICG. A potential drawback of this rapid spread is that lymph nodes are excised that are not truly sentinel nodes (i.e. no first-draining lymph nodes), leading to overtreatment and accompanying co-morbidity. To solve this problem, the molecular size of ICG can be increased by linking it to human serum albumin, creating a noncovalent bond.[32] In addition to reducing the spread of ICG, the binding

to human serum albumin also increases the brightness of the fluorescent signal as it reduces quenching of the fluorescent molecules (i.e. reduction of fluorescent signal due to photons being absorbed by nearby fluorescent molecules).

Conclusion

The current meta-analysis provided data that favor the use of radiocolloid solely or radiocolloid combined with a blue dye for the identification of the SLN. Performing SLNB with radiocolloid solely is the technique of choice for experienced surgeons, since blue dye has multiple disadvantages. Moreover, NIRF imaging with ICG as a fluorescent dye seems a promising technique, although hurdles like the limited penetration depth of optical signals still reduces general applicability of the technique.

Table 1. Identification rates reported in studies for sentinel lymph node biopsy using blue dye, radiocolloid or a combination of these two in breast carcinoma patients.

Study	Year of publication	Technique	No of patients	Mean SLN	Identification rate (%)	False negative rate (%)	Follow up in mean (*) or median (^) months
Giuliano[49]	1997	Blue	107		93·5%	0·0%	
Barnwell[50]	1998	Combi	42	1·0	90·0%	0·0%	
Crossin[51]	1998	Radio	50	2·0	84·0%	2·4%	
Flett[52]	1998	Blue	68		82·4%	5·4%	
Kapteijn[53]	1998	Blue	30	1·4	86·7%		
Krag[3]	1998	Radio	443	2·6	93·2%	11·0%	
Ratanawichitrasin[54]	1998	Blue	40	1·6	87·5%		
Snider[55]	1998	Radio	80	2·2	87·5%	7·0%	
Jaderborg[56]	1999	Combi	79	1·5	81·0%	5·0%	
Kollias[57]	1999	Blue	19		94·7%		
		Radio	51		68·6%		
		Combi	47		89·0%	6·5%	
Moffat Jr.[58]	1999	Radio	70	2·1	98·6%		
Morgan[59]	1999	Blue	44		72·7%	16·7%	
Morrow[39]	1999	Blue	50		88·0%		
		Combi	42		86·0%		
Veronesi[60]	1999	Radio	376		98·7%	6·7%	
		Combi	54		68·5%		
Winchester[61]	1999	Radio	180	3·1	90·0%	2·2%	
Canavese[62]	2000	Blue	55		65·5%	23·0%	
		Combi	48		94·0%	12·5%	

Cox[63]	2000	Blue[a]	1147	2·1	80·3%		
		Radio[a]	1147	2·1	88·6%		
		Combi	1147	2·1	96·0%	1·0%	
Doting[64]	2000	Combi	136	1·7	93·0%	5·1%	
Fraile[65]	2000	Radio	132	2·0	96·2%	4·0%	
Lauridsen[38]	2000	Blue[a]	80		90·0%		
		Radio[a]	80		92·5%		
		Combi	80	2·0	98·0%	0·0%	
Liu[66]	2000	Combi	41	1·5	93·0%		
Rodier[67]	2000	Blue	74		82·4%	8·0%	
Tsugawa[68]	2000	Blue[a]	48		75·0%		
		Radio[a]	48		66·6%		
		Combi	48		90·0%	4·7%	
Derossis[22]	2001	Blue[a]	2000		82·9%		
		Radio[a]	2000		90·1%		
		Combi	2000		97·0%		
Feggi[69]	2001	Radio	73	1·0	100·0%		
Mateos[70]	2001	Blue[a]	65		72·3%	16·7%	20^
		Radio[a]	80	1·2	91·2%	17·0%	
		Combi	80		91·0%		
Sato[71]	2001	Radio	75	1·9	98·7%		
Simmons[72]	2001	Blue	30	1·8	90·0%		
Tafra[73]	2001	Combi	535	1·6	87·0%	5·2%	
Tanis[74]	2001	Combi	60	2·2	97·0%		8^
Xavier[75]	2001	Blue	6		100·0%		
		Combi	50		100·0%	2·0%	
Watanabe[76]	2001	Radio	87	2·0	100·0%		
Feezor[77]	2002	Radio	118	1·8	98·3%		
Kern[78]	2002	Combi	187	2·4	98·0%	0·0%	
Jastrzebski[79]	2002	Combi	123		88·0%		
Shimazu[80]	2002	Blue[a]	62	1·5	79·9%		
		Radio[a]	93		92·5%		
		Combi	93	2·0	96·0%	5·6%	
Nos[81]	2003	Blue	324	2·1	85·5%	11·1%	
Reitsamer[82]	2003	Combi	157	2·0	99·0%		
Simmons[83]	2003	Blue[a]	112		92·9%		84*
		Radio[a]	112		89·3%		

		Combi	112		96·0%		
Eldrageely[84]	2004	Blue[a]	164		90·2%		
		Radio[a]	164		94·5%		
		Combi	164	1·5	98·0%		
Gray[85]	2004	Combi	546	1·3	99·0%		
Lin[33]	2004	Blue	150		82·7%		
		Radio	94		96·8%		
		Combi	76		97·0%		
King[27]	2004	Blue[a]	1719		84·3%		
		Radio[a]	1719		97·3%		
		Combi	1719		99·0%		
Nour[86]	2004	Blue	54		83·3%	0·0%	
Radovanovic[87]	2004	Blue	50	1·7	68·0%	18·0%	
		Combi	150		83·0%	4·5%	
Wessem, van[88]	2004	Blue[a]	132		84·1%		32*
		Radio[a]	132		95·5%		
		Combi	132	1·4	97·0%	6·0%	
Hung[89]	2005	Blue	57	1·8	86·0%	5·0%	
		Combi	61	2·1	100·0%	0·0%	
Syme[90]	2005	Blue	93	1·3	95·7%	20·8%	
		Combi	257	1·8	89·0%	2·8%	
Teal[91]	2005	Blue[a]	99	2·2	81·8%		
		Radio[a]	99	2·2	98·0%		
		Combi	99	2·2	99·0%		
Argon[25]	2006	Blue[a]	100	1·4	88·0%		
		Radio[a]	100	1·8	96·0%		
		Combi	100	1·6	98·0%		
Golshan[92]	2006	Blue	141	2·0	96·5%		
Lo[93]	2006	Radio	175	1·1	94·3%	2·5%	
		Combi	758	2·0	89·0%	5·8%	
Povoski[94]	2006	Radio	400		95·0%		
		Combi	267		97·0%		
Takei[95]	2006	Blue[a]	308	1·7	96·8%		33^
		Radio[a]	308	1·4	95·8%		
		Combi	308	1·9	99·0%		
Zaman[96]	2006	Combi	32		97·0%	0·0%	
Kargozaran[97]	2007	Blue[a]	124	1·6	92·7%		23^
		Radio[a]	124	1·7	97·6%		

		Combi	124		98·0%		
Krag[37]	2007	Combi	5536	1·3	97·0%		
Nathanson[98]	2007	Blue[a]	600		89·3%		
		Combi	600		96·0%		
Rodier[36]	2007	Blue[a]	449		94·7%		48^
		Radio[a]	449		97·1%		
		Combi	449	1·8	99·0%		
Varghese[99]	2007	Blue	173	1·5	96·5%	3·7%	
		Combi	156	2·5	99·0%	2·5%	
Yen[100]	2007	Radio	213	3·5	97·2%	4·4%	
Bines[26]	2008	Radio	208	2·0	93·8%		
		Combi	167	1·9	96·0%		
Mudun[101]	2008	Radio	228		97·4%	3·1%	
Thompson[40]	2008	Combi	236	1·6	96·0%		
Zakaria[102]	2008	Blue[a]	401		88·0%		
		Combi	401		100·0%		
Climaco[103]	2009	Combi	46		76·0%		
Koukouraki[104]	2009	Blue[a]	250		94·4%		
		Combi	250		100·0%		
Mathelin[105]	2009	Blue[a]	100	2·7	65·0%		28*
		Radio[a]	100	2·7	94·0%		
		Combi	100	2·7	99·0%		
Hayashida[106]	2010	Blue[a]	640	2·4	79·7%		
		Radio[a]	640	2·4	94·7%		
		Combi	640	2·4	98·0%		
Kang[23]	2010	Radio	1353	2·9	98·4%		
		Combi	2049	2·7	98·0%		
Krikanova[107]	2010	Blue	332		94·6%		
Narui[108]	2010	Blue	234	3·4	99·6%		54^
Straver[109]	2010	Blue[a]	1953		88·3%		
		Radio[a]	1953		95·8%		
		Combi	1953		97·0%		
Yararbas[110]	2010	Radio	200		98·0%		
Iida[111]	2011	Combi	258		99·0%	7·7%	
Mieog[112]	2011	Blue[a]	30	1·5	83·3%		
		Radio[a]	30	1·5	100·0%		

[a]Blue dye and radiocolloid were used in the same procedure. SLN identification rates were recorded separately. BC: Breast Carcinoma. SLN: Sentinel lymph node

Table 2. Identification rates reported in studies for sentinel lymph node biopsy using blue dye, radiocolloid or a combination of these two in melanoma patients.

Study	Year of publication	Technique	no patients	mean SLN	Identification rate (%)	False negative rate (%)	Follow up in mean (*) or median (^) months
Thompson[113]	1995	Combi	118		87·0%	5·2%	
Albertini[114]	1996	Blue[a]	106		69·5%		
		Combi	106	1·9	96·0%	0·0%	60*
Thompson[115]	1997	Blue[a]	21		83·0%		
		Radio[a]	21		87·2%		
		Combi	21	2·2	91·3%		
Wells[116]	1997	Blue[a]	58	2·3	67·0%		
		Combi	36		95·0%	0·0%	12^
Bartolomei[117]	1998	Blue[a]	25		72·0%		
		Radio[a]	25		100·0%		
		Combi	25	1·5	100·0%	0·0%	11*
Bedrosian[118]	1999	Blue	23		74·0%		
		Combi	80	1·8	94·7%		
Bostick[119]	1999	Blue[a]	87		95·1%		
		Radio[a]	87		92·3%		
		Combi	87	1·6	97·6%	0·0%	16*
Gennari[120]	1999	Combi	133	1·6	99·0%	3·8%	19^
Gershenwald[121]	1999	Combi	612	1·7	95·0%		40^
Morton[122]	1999	Blue	453		94·9%		
		Combi	727		98·3%	3·7%	
Jansen[123]	2000	Combi	200	2·2	99·5%	3·9%	
Jansen[124]	2000	Combi	30	2·3	90·0%	9·1%	23*
Landi[125]	2000	Blue	25	1·5	88·0%		
		Combi	425	1·6	99·5%	1·2%	18^
Oliveira Filho[126]	2000	Blue[a]	64		76·0%		
		Radio[a]	64		97·0%		
		Combi	64	1·4	100·0%	1·9%	11^
Temple[127]	2000	Combi	56	2·2	98·0%	4·5%	12^
Tremblay[128]	2000	Radio	36	2·0	97·2%	0·0%	14^
Villa[129]	2000	Blue[a]	88	1·9	94·3%		
		Radio[a]	49		98·0%		

		Combi	49		98·0%		
McMasters[130]	2001	Blue[a]	1184		69·0%		
		Combi	1184	2·4	99·7%	2·0%	
Medina-Franco[131]	2001	Blue[a]	38		68·4%		
		Radio[a]	38		89·5%		
		Combi	38		97·0%	2·9%	15*
Neubauer[132]	2001	Radio	41	1·3	95·1%		
Rasgon[133]	2001	Blue[a]	24		66·7%		
		Radio[a]	24	3·0	91·7%		
		Combi	24		92·0%	10·5%	18*
Tavares[134]	2001	Radio[a]	19		95·0%		
		Combi	19	1·7	95·0%	0·0%	
Eicher[135]	2002	Blue[a]	43		27·9%		
		Radio[a]	43		90·7%		
		Combi	43	3·6	98·0%	0·0%	
Ferrone[136]	2002	Combi	126	2·0	100·0%		25*
Patel[137]	2002	Blue[a]	48		73·0%		
		Radio[a]	56	2·4	93·0%		
		Combi	48		96·0%	1·9%	20^
Chao[138]	2003	Blue[a]	254	2·1	59·1%		
		Radio[a]	321	2·8	97·0%	2·2%	16^
Estourgie[139]	2003	Combi	250	2·3	100·0%	3·2%	72^
Schmalbach[140]	2003	Blue	80	2·2	96·0%	4·5%	25^
Vidal-Sicart[141]	2003	Blue[a]	315		70·1%		
		Radio[a]	435	1·8	98·8%	1·9%	26*
Weiss[142]	2003	Blue[a]	30		67·0%		
		Radio[a]	30		100·0%		
		Combi	30	1·9	100·0%		
Alex[143]	2004	Blue[a]	16		81·3%		
		Radio[a]	43		97·6%	2·6%	82*
		Combi	16		100·0%		
Chakera[144]	2004	Radio[a]	241	2·6	98·0%		
		Combi	194	1·7	100·0%	1·1%	15^
Gipponi[145]	2004	Blue	39	1·4	89·7%		
		Combi	126	1·9	100·0%	4·4%	17^
Rossi[146]	2004	Combi	1313	2·0	99·3%	3·5%	31^

Topping[30]	2004	Combi	347	2·1	99·0%	1·7%	60^
Shpitzer[147]	2004	Blue	8	1·7	88·0%		
		Combi	22	2·0	96·0%	3·8%	31^
Carlson[148]	2005	Radio[a]	132	2·1	96·9%	6·4%	35*
MacNeill[149]	2005	Combi	44		93·0%	0·0%	22*
Doting[150]	2006	Blue[a]	36		63·9%		
		Radio[a]	36		91·7%		
		Combi	36	2·7	92·0%	6·9%	54^
Gad[151]	2006	Combi	278	2·2	98·0%	2·5%	31^
Lin[152]	2006	Combi	114	4·0	97·0%	4·0%	84*
Oliveira Filho[153]	2006	Blue	47	1·5	100·0%		
		Radio	47	1·6	100·0%		
		Combi	94	1·6	100·0%	2·7%	20^
Cecchi[154]	2007	Combi	30	1·3	100·0%	21·1%	27*
Kilpatrick[155]	2007	Blue[a]	316		88·4%		
		Radio[a]	316		97·4%		
		Combi	316	2·8	97·8%	4·7%	19*
Koskivuo[156]	2007	Combi	305	2·4	97·0%	2·0%	21*
Teltzrow[157]	2007	Radio	106	2·3	89·0%	9·0%	47*
Gomez-Rivera[158]	2008	Combi	111	3·2	100·0%	5·6%	34^
Liu[159]	2008	Blue[a]	159		60·2%		
		Combi	159		100·0%		
Mattsson[160]	2008	Combi	422	2·1	97·0%	4·2%	12^
Roulin[28]	2008	Combi	327	2·0	99·1%	2·8%	34*
Kelly[161]	2009	Combi	40		68·0%	9·5%	40^
Kovacevic[162]	2009	Blue[a]	40		95·0%		
		Radio[a]	40		100·0%		
		Combi	40	1·9	100·0%	16·7%	18*
Koskivuo[163]	2011	Combi	423		96·0%	2·5%	36^
Leong[164]	2011	Radio[a]	47	2·3	98·9%		
Liu[165]	2011	Combi	571	3·4	100·0%		
Neves[166]	2011	Blue[a]	93	1·8	47·0%		
Noro[167]	2011	Blue	47		83·0%		
		Combi	74		95·9%		
Rughani[29]	2011	Combi	697	1·9	99·7%	3·0%	46^

[a]Blue dye and radiocolloid were used in the same procedure. SLN identification rates were recorded separately.

SLN: Sentinel lymph node

Table 3. Identification rates reported in studies combining radiocolloid, vital blue dye, and/or indocyanine green in breast carcinoma and melanoma patients.

Study	Tumor type	Year of publication	Technique	no patients	mean SLN	Identification rate (%)	False negative rate (%)	Follow up in mean (*) or median(^) months
Motomura[168]	Breast carcinoma	1999	Green	150	1·7	76·7%	1·1%	
Motomura[169]	Breast carcinoma	2001	Green	93	1·8	83·9%	1·9%	
			G+R	138		94·9%	0%	
Kitai[17]	Breast carcinoma	2005	Green	18	2·8	94·4%		
Tagaya[170]	Breast carcinoma	2008	Green[a]	25	5·4	100%		6*
			Blue[a]	25	2·3	92·0%		
Murawa[171]	Breast carcinoma	2009	G+R	30		96·7%	0·1%	
Tanaka[172]	Melanoma	2009	Radio[a]	4		100·0%		
			Green[a]	4		100·0%		
			G+R	4		100·0%		
Hirche[173]	Breast carcinoma	2010	Green	43	2	97·7%	6·6%	
Abe[174]	Breast carcinoma	2011	Green[a]	128	3·1	100%		
			Blue[a]	128		65·6%		
Aoyama[175]	Breast carcinoma	2011	Green	312	3·4	100%	1·7%	49^
Tagaya[176]	Breast carcinoma	2011	Green	50	3·7	100%		6*
Fujisawa[177]	Melanoma	2011	Blue[a]	6	2	100·0%		
			Radio[a]	5	1·8	100·0%		
			Green[a]	6	2·2	100·0%		
			G+R+B	6	2·2	100·0%		
Namikawa[178]	Melanoma	2011	Blue[a]	49	2	85·7%	5·6%	
			Radio[a]	49	2·5	95·9%	5·6%	
			Green[a]	49	4	61·2%	3·89%	
			G+R+B	49	4	100·0%	0%	20^
Hirche[179]	Breast carcinoma	2012	Green	47	2	97·9%	5·3%	
Polom[180]	Breast carcinoma	2012	G+R	49	2·3	95·9%		
Brouwer[181]	Melanoma	2012	Blue[a]	7	1·3	71·4%		
Fujisawa[182]	Melanoma	2012	Blue[a]	15	1·7	93·0%		
			Radio[a]	15	1·7	100·0%		
			Green[a]	15	2	100·0%		

			G+R+B	15	2	100·0%		
Polom[183]	Melanoma	2012	Radio[a]	10		100·0%		
			Green[a]	10		100·0%		
			G+R	10	2·3	100·0%		
Stoffels[184]	Melanoma	2012	Radio[a]	22		100·0%		
			Green[a]	22		100·0%		
			G+R	22	2·8	100·0%	0%	1·3*
Uhara[185]	Melanoma	2012	Blue[a]	562		92·3%		
			Radio[a]	562		96·4%		
			Green[a]	67		100·0%		
			G+R+B[b]	562		97·9%		

[a]Blue dye, radiocolloid, and/or indocyanine green were used in the same procedure. SLN identification rates were recorded separately. [b]Indocyanine green was used in 67 out of 562 patients.

G+R+B: SLNB using green dye + radiocolloid + blue dye. SLN: Sentinel lymph node.

Table 4. Pooled identification rate for sentinel lymph node biopsy for all studies, breast carcinoma studies, and melanoma studies per technique.

		All		**Breast carcinoma**		**Melanoma**	
Technique	Period	Pooled value	95%CI	Pooled value	95%CI	Pooled value	95%CI
Blue dye alone	Whole period	84%	83-86%	85%	83-88%	84%	83-84%
	1992-2000	84%	81-87%	81%	76-86%	91%	91-92%
	2001-2006	81%	78-84%	86%	82-91%	72%	61-84%
	2007-2012	88%	85-91%	87%	84-91%	89%	82-96%
Radio colloid alone	Whole period	95%	95-96%	94%	93-95%	99%	99-99%
	1992-2000	92%	90-93%	88%	84-91%	100%	100-100%
	2001-2006	95%	94-97%	96%	94-97%	97%	96-97%
	2007-2012	98%	97-98%	97%	96-98%	100%	99-100%
Combination radio colloid and blue dye	Whole period	96%	96-97%	95%	94-95%	98%	98-98%
	1992-2000	95%	94-95%	91%	88-94%	97%	97-98%
	2001-2006	97%	96-97%	96%	95-96%	99%	98-99%
	2007-2012	98%	97-98%	97%	96-98%	99%	98-99%
ICG	Whole period	99%	98-99%	95%	95-96%	100%	100-100%
	1992-2000	77%*	76-77%	77%*	76-77%	No studies	
	2001-2006	89%	79-99%	89%	79-99%	No studies	
	2007-2012	100%	100-100%	100%	100-100%	100%	100-100%
Combination radio colloid and ICG	Whole period	99%	99-100%	96%	95-97%	100%	100-100%
	1992-2000	No studies		No studies		No studies	
	2001-2006	95%*	95-95%	95%	95-95%	No studies	
	2007-2012	100%	100-100%	96%	96-97	100%	100-100%

*Derived from only one study. ICG: Indocyanine green.

Table 5. Pooled false negative rate for sentinel lymph node biopsy in all studies, breast carcinoma studies, and melanoma studies per technique.

		All		Breast carcinoma		Melanoma	
Technique	Period	Pooled value	95%CI	Pooled value	95%CI	Pooled value	95%CI
Blue dye alone	Whole period	3·4%	3·0-3·8%	3·2%	2·8-3·6%	6·1%*	5·2-7·1%
	1992-2000	3·4%	1·1-5·8%	3·4%	1·1-5·8%	No studies	
	2001-2006	3·5%	1·7-5·3%	3·5%	1·7-5·3%	No studies	
	2007-2012	4·5%	1·3-7·6%	2·9%*	2·7-3·1%	6·1%*	5·2-7·1%
Radio colloid alone	Whole period	2·6%	1·9-3·3%	2·2%	1·8-2·6%	3·4%	2·6-4·2%
	1992-2000	1·6%	0·7-2·4%	2·0%	1·3-2·7%	0·5%	0-1·3%
	2001-2006	2·7%	0·7-4·7%	2·7%	0·7-4·7%	No studies	
	2007-2012	4·5%	3·0-6·0%	2·3%	1·5-3·0%	6·9%	5·5-8·3%
Combination radio colloid and blue dye	Whole period	2·1%	1·9-2·3%	1·5%	1·3-1·7%	2·6%	2·3-2·9%
	1992-2000	1·9%	1·7-2·1%	1·9%	1·5-2·2%	1·9%	1·6-2·3%
	2001-2006	1·9%	1·5-2·3%	1·1%	0·7-1·6%	2·5%	1·9-3·1%
	2007-2012	3·7%	2·8-4·5%	1·7%	0-3·4%	3·4%	3·0-3·8%
ICG	Whole period	4·4%	3·3-5·4%	2·8%	2·3-3·3%	14·3%*	12·9-15·7%
	1992-2000	2·7%*	2·5-2·9%	2·7%*	2·5-2·9%	No studies	
	2001-2006	4·3%*	3·9-4·7%	4·3%*	3·9-4·7%	No studies	
	2007-2012	5·2%	2·6-7·8%	2·5%	2·3-2·7%	14·3%*	12·9-15·7%
Combination radio colloid and ICG	Whole period	0·1%	0·1-0·1%	0·1%	0·1-0·1%	0%	0-0
	1992-2000	No studies		No studies		No studies	
	2001-2006	0%	0-0%	0%	0-0%	No studies	
	2007-2012	0·1%	0·1-0·1%	3·3%	0-9·8%	0%	0-0

*Derived from only one study. ICG: Indocyanine green.

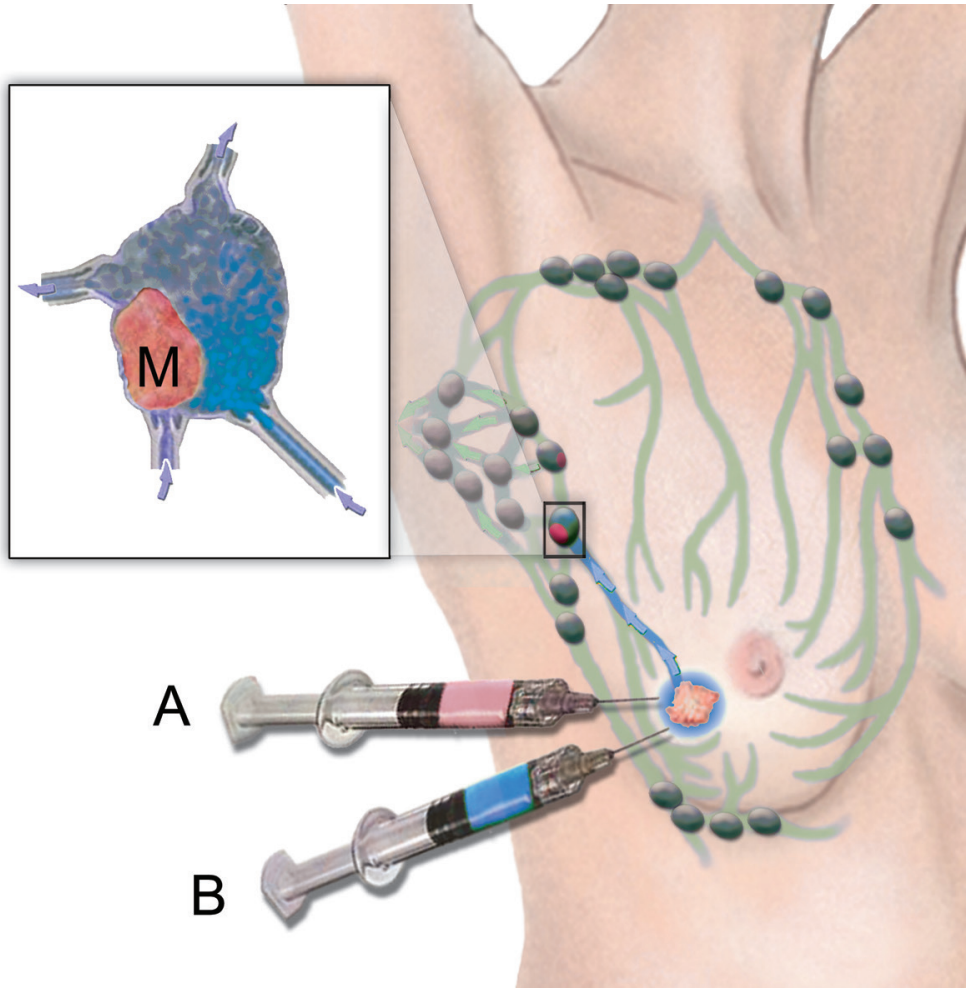

Figure 1

Schematic overview of the sentinel lymph node procedure in a breast cancer patient. Before surgery, the patient is injected peritumorally with a radiocolloid (A) and/or vital blue dye (B). Both substances flow along with the lymphatic fluid and accumulate in the sentinel lymph node. During surgery, the surgeon localizes the sentinel lymph node by visual inspection (blue dye) and a gamma probe (radiocolloid). After excision, the sentinel lymph node is evaluated for the presence of metastasis (M) by a pathologist.

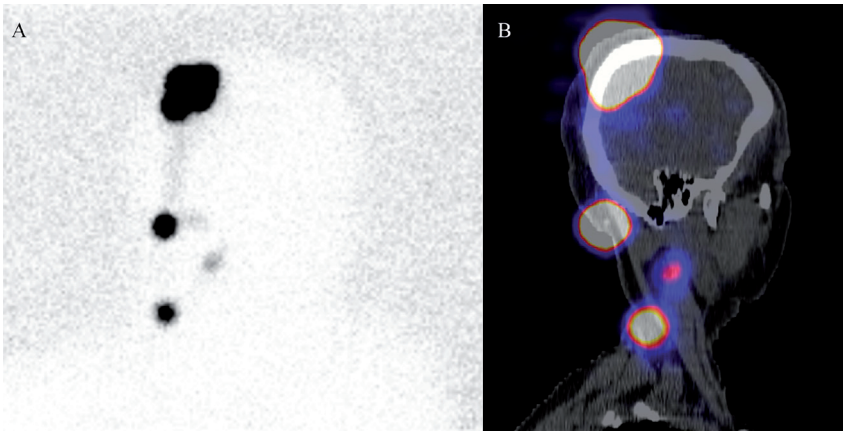

Figure 2. Lymphoscintigraphy (**A**) and SPECT/CT (**B**) images of a patient with a primary melanoma on the scalp and lymph nodes in the neck.

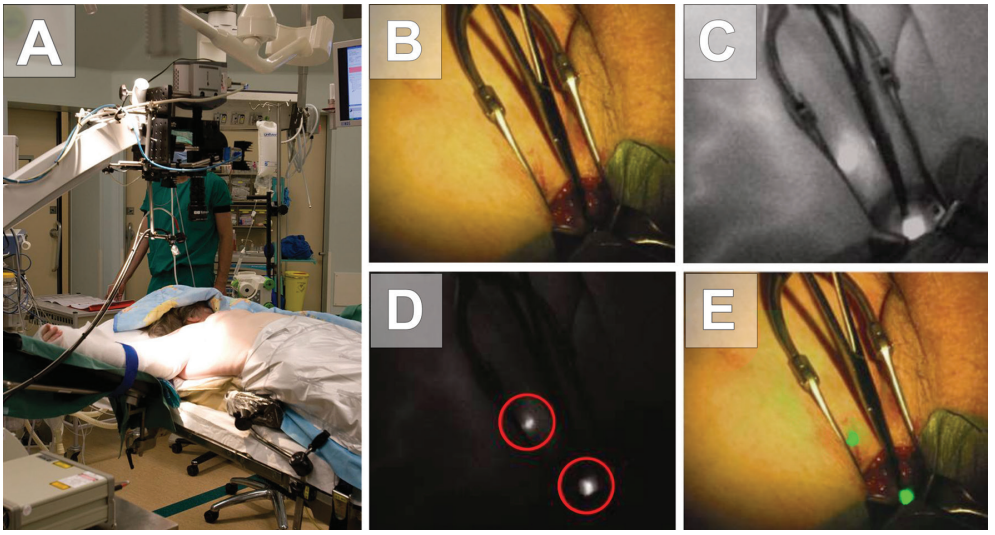

Figure 3. Intraoperative sentinel lymph node localization using a near-infrared fluorescence (NIRF) imaging device in combination with indocyanine green (ICG). The NIRF imaging device is positioned above the patient (**A**) and can be covered in sterile drapes for intraoperative use (not shown). A color image of the surgical field is shown, representing the axillary region of a breast cancer patient (**B**). After peritumoral injection with ICG, the NIRF imaging device is applied to visualize the sentinel lymph node (SLN) and corresponding lymphatic vessels (**C**). By adjusting the threshold for the fluorescent signal, the SLNs (indicated by red circles) become clearly visible (**D**). For anatomical positioning of the fluorescent signal, the signal can be superposed on a color image of the surgical field as a contrasting pseudo-color (e.g. green; **E**).

Supplementary file 1

Search method and inclusion/exclusion criteria

A computer-aided search was conducted using the following databases: PubMed/Medline; Database of Abstracts of Reviews of Effects (DARE) database; Embase; and Cochrane. PubMed was searched as the primary source for scientific articles. Search terms included: “breast carcinoma”, “breast neoplasm”, or “melanoma”; “sentinel lymph nodes“; “lymphatic mapping”, “lymph node mapping”; “radioisotopes”; “radiocolloid”; “technetium”; “Patent blue violet”; “Methylene blue”; “Isosulfan blue”; and “Blue dye”.

We augmented our computerized literature search by manually reviewing the reference lists of identified studies and relevant reviews. In addition, Google was searched as a complementary source for related studies. Two reviewers (MGN/RGP) independently assessed the eligibility of all identified studies by checking titles and abstracts. Studies that clearly did not meet the inclusion criteria were excluded. Full text articles were retrieved of potentially relevant references. If there was any disagreement between the readers, a consensus was reached by discussion or by consulting a third reviewer (GMD/HJH). Data from the articles was retrieved and imported into a data abstraction spreadsheet (Microsoft Excel SP3; Microsoft Corp., Redmond, WA) specifically designed for the review.

Criteria for inclusion were: original study group, intraoperative SLN identification in early stage (stage I or II) breast carcinoma and or melanoma using radiocolloid tracer, blue dye, ICG, or a combination of a radiocolloid tracer with a blue or ICG. Criteria for exclusion were: studies not in the English language; studies that were published before 1992; case reports, reviews, and editorials; studies that did not describe the SLN identification rate (IR); and articles lacking a clear definition of the SLN. Duplicate articles on the same group or follow-up studies with an included subset of previously reported patients were also excluded. The final decision regarding inclusion was based on the full article.

The SLN is generally defined as the initial lymph node that directly drains the lymph fluid from the site of the primary lesion.[186] Despite a good understanding of the theoretical definition of a SLN, there is no consensus on the clinical definition of SLNs detected with a radiotracer.[24] The SLN has been described as the hottest node, first node visualized on lymphoscintigraphy, and the node with radioactivity greater than twice or thrice the background radioactivity.[186,187] Data from the Sunbelt Melanoma Trial showed that resection of all blue-stained nodes and all nodes with more than 10% of the hottest node’s radioactivity (10% rule) was associated with a low estimated false-negative rate.[130,188] In this systematic review, articles were included only when SLNs were defined as all blue nodes and all hot nodes that had radioactive counts greater than the background or when the 10% rule was applied for hot nodes.

Supplementary file 2
QUADAS quality assessment tool

The following core QUADAS items were assessed in the current review to assess methodological quality:

1. Was the spectrum of patients representative of the patients who will receive the test in practice?

- Yes: at least 80% of patients had early stage breast carcinoma or melanoma.
- No: less than 80% of patients had early stage disease.
- Unclear: stage of disease was not clear from the available information; or, in studies which included both early and late disease, we could not clearly distinguish between information on patients with early disease and those with late disease.

2. Where selection criteria clearly described?

- Yes: all relevant information regarding how participants were selected for inclusion in the study has been provided.
- No: selection criteria were not specified.
- Unclear: selection criteria are only partially reported.

3. Is the reference standard likely to correctly classify the target condition?

- Yes: reference standard is carried out adequately to correctly classify the target condition.
- No: reference standard has not been carried out adequately to correctly classify the target condition.
- Unclear: there is insufficient information given to assess whether reference standard had been carried out adequately (e.g. no mention of site or number of SLN identified).

4. Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?

- Because the reference standard and index test are applied simultaneously in SLN identification, all items will be scored 'yes'.

5. Was the execution of the index test described in sufficient detail to permit replication of the test?

- Yes: for SLN biopsy using blue dye, description of the execution of the test should include type and amount of tracer substance used, site and timing of application of tracer substance and method of detection of tracer substance.
- No: the description does not include details as stated above.
- Unclear: it is unclear from the available description as to whether the test can be replicated.

6. Was the execution of the reference test described in sufficient detail to permit its replication?

- Yes: for SLN biopsy using radiocolloid tracer, the description includes type and amount of tracer substance used, site and timing of application of tracer substance, method of detection of tracer substance, and use of preoperative scintigraphy.
- No: the above details were not clearly stated.
- Unclear: the description is not adequate to allow replication of the test.

7. Were the reference standard results interpreted without knowledge of the results of the index test?

- Yes: identification of SLNs using the reference standard test was interpreted without knowledge on SLN identification using blue dye.
- No: identification of SLNs using the reference standard test was interpreted with knowledge on SLN identification using blue dye.
- Unclear: insufficient details given as to whether the reference standard results were interpreted with or without knowledge of the results of the index test.

8. Were the index test results interpreted without knowledge of the results of the reference standard?

- Yes: the identification of SLNs using blue dye was interpreted without knowledge (blind) on SLN identification using the reference standard.
- No: the identification of SLNs using blue dye was interpreted with knowledge (blind) on SLN identification using the reference standard.
- Unclear: no description of when and how the index tests results are interpreted.

9. Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?

- Yes: the same clinical information was available when the test results were interpreted as would be available when the test is used in practice such as age of patients, clinical history, any related investigation results.
- No: different clinical information, or more or less clinical data, were available; e.g. if tests were interpreted without knowledge of standard clinical data as stated above.
- Unclear: insufficient details given as to what clinical information was available.

10. Were uninterpretable/intermediate test results reported?

- Yes: all uninterpretable results were reported; e.g. if it was not obvious whether a patient had a positive test result.
- No: not all uninterpretable results were reported.
- Unclear: insufficient information to determine whether all uninterpretable results were reported.

11. Were withdrawals from the study explained?

- Yes: all withdrawals from the study were explained.
- No: not all withdrawals were explained.
- Unclear: insufficient information as to whether all withdrawals were explained.

12. Did the whole or a random selection of the sample, receive verification using a reference standard of diagnosis?

- Yes: reference standard (intraoperative SLN identification using radiocolloid tracer) has been carried out in the whole population.
- No: reference standard has not been carried out in the whole population.
- Unclear: no clear information about the proportion of patients receiving verification using the reference standard.

13. Did patients receive the same reference standard regardless of the index test result?

- Yes: all the patients received the same reference standard regardless of the index test (intraoperative use of blue dye) result.
- No: not all the patients receive the same reference standard regardless of the index test result.
- Unclear: insufficient information to assess whether all the patients received the same reference standard.

14. Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?

- Yes: it is clear that the index test did not form part of the reference standard.
- No: it appears that the index test formed part of the reference standard.
- Unclear: information is not reported by the study.

15. Was the follow-up adequate and false negative clearly reported?

- Yes: The follow-up was adequate (at least 12 months or additional lymph dissection) and the false negative rate was clearly reported.
- No: The follow-up was not adequate and the false negative was not clearly reported.
- Unclear: No clear information was available on the follow-up and false negative rate.

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Chapter 6

Deep lymph node metastases in the groin significantly affect prognosis, particularly in sentinel node positive melanoma patients

M.G. Niebling, K.P. Wevers, A.J.H. Suurmeijer, R.J. van Ginkel, H.J. Hoekstra

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Abstract

Background

In order to define patients eligible for only a superficial groin dissection or a combined superficial and deep groin dissection, this study aimed to determine the incidence of deep lymph node metastases (LNM) in melanoma patients metastasized to the groin, to identify patient and melanoma factors that predict deep nodal involvement, and to analyze the impact of deep nodal involvement on survival and recurrence.

Methods

Patients who underwent a combined superficial (inguinal) and deep (iliac and obturator) Completion or Therapeutic lymph node dissection (CLND or TLND) of the groin between 1994 and 2012 were analyzed.

Results

Deep LNM were present in 8 of 62 CLND patients (13%) and in 21 of 67 TLND patients (31%). More than 3 superficial LNM was the only independent predictor for deep LNM in both CLND and TLND patients. The 5-year Melanoma Specific Survival (MSS) for CLND and TLND patients with deep LNM was 14.3% and 16.6%, and was significantly worse (HR:3.39, 95%CI:1.34-8.58, P=0.010 and HR:2.01, 95%CI:1.04-3.88, P=0.039) compared to CLND and TLND patients without deep LNM (5-year MSS: 54.1% and 37.2%).

Conclusions

The present study showed that LNM in the deep area of the groin are fairly common in both CLND and TLND patients and significantly affect prognosis, especially in CLND patients. The presence of deep nodal involvement can only be predicted by the number of superficial lymph node metastases.

Introduction

Lymph node metastases (LNM) are common in melanoma patients. They are usually detected clinically by the patient or clinician or using a Sentinel Lymph Node Biopsy (SLNB).[1] The SLNB is a minimally invasive staging procedure recommended by the American Joint Committee on Cancer (AJCC)[2] and is widely accepted and recommended by the Society of Surgical Oncology (SSO) and the American Society of Clinical Oncology (ASCO) in patients with ≥1mm melanoma.[3]

According to the incubator hypothesis, the primary melanoma spreads primarily to sentinel nodes in the regional lymph basin, where the metastatic melanoma cells may survive and grow slowly or remain latent before, in some patients, spreading to distant sites.[4] Hypothetically, if the regional metastatic disease could be removed prior to systemic spread, the patient would be cured.

Melanoma patients with regional metastases detected by SLNB are generally treated with a Completion Lymph Node Dissection (CLND) or alternatively monitored with ultrasound in the Multicenter Selective Lymphadenectomy Trial II (MSLT-II).[5] Patients with palpable nodal (Stage IIIB/C) are first staged using FDG-PET/CT (Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography), when no distant metastatic disease is encountered these patients are treated by a so called Therapeutic Lymph Node Dissection (TLND) with or without adjuvant systemic and/or radiation therapy.[6,7]

Combined superficial & deep LND for patients with LNM in the groin showed to have better survival rates and a decreased risk for local failure compared to solely superficial LND.[8-10] However, deep LND could increase the risk for postoperative morbidity including wound complications and edema, while in many patients the deep lymph nodes are not involved.[11]. Therefore, it would be appropriate to omit deep lymph node dissection if one could predict a low risk for deep LNM. This might reduce the risk for morbidity without increasing the risk of local failure.[12,13]

In addition, a subgroup of patients might benefit from additional adjuvant treatment including systemic and/or radiation therapy.[6,7]

The aim of the present study was to investigate the incidence of deep LNM in patients who underwent CLND or TLND of the groin and to find predictive factors for the presence of deep LNM. Furthermore, the impact of the presence of deep LNM on survival and recurrence was analyzed.

Materials and Methods

Patients

Melanoma patients who underwent combined superficial (inguinal) and deep (iliac and obturator) LND of the groin at the University Medical Center Groningen (UMCG), the Netherlands, between 1994 and 2012 were included. Patients were only included when the pathology report was clear on the superficial or deep (inguinal or iliac/obturator) localization of all dissected LNs. Patients with clinically detected LNM underwent combined superficial and deep TLND. Patients with a positive hematoxylin and eosin SLNB underwent either combined superficial and deep CLND or were monitored with nodal ultrasound, since the majority of these patients were included in the MSLT-II trial. Only the patients

that underwent CLND were included in this study. When the metastasis in the SLN was ≤1.0mm or only positive using immunohistochemistry a superficial CLND was performed and therefore these patients were excluded from the study.S

Surgical procedure

The surgical procedure is fully described in the Atlas of Advanced Operative Surgery.[14] The long-term morbidity of the procedure is limited.[11,15,16] However, the morbidity increases if patients are treated with adjuvant radiotherapy.[12,17-19]

Pathology

All LNs were marked according to their anatomical origin (inguinal, iliac or obturator). For histopathologic analysis of the completely embedded LNs, 4 µm thick sections were stained with hematoxylin and eosin (H&E). The number of dissected LNs, size of the largest LN and presence of extranodular growth of the LNM were determined.

Data analysis

Characteristics of the patient (sex and age), primary melanoma (Breslow thickness, Clark level, ulceration, mitotic rate, histologic subtype, and primary disease site) and LNM (time to metastasis, extranodal growth pattern, total number of superficial and deep nodes, number of involved superficial and deep nodes, involved/total LNs (L/N) ratio, size of the largest nodal metastasis) were recorded and analyzed.

Both the Melanoma Specific Survival (MSS), with death due to melanoma as event after LND, and Disease Free Period (DFP), with any recurrence as event after LND, were calculated for CLND and TLND patients. Recurrences in the LNs of the groin and distant recurrences were recorded for all CLND and TLND patients.

The Chi square test was used to calculate whether there was a significant difference between CLND and TLND patients using a significant level of $p<0.05$ in categorical variables. For continuous variables the Mann-Whitney U test and the Independent T-test were used, depending on the distribution of the variable. Kaplan Meier curves for MSS and DFP were constructed and differences were assessed using the log-rank test. Cox regression analysis was used to calculate survival and recurrence differences, and hazard ratios (HR) for the presence of deep LNM. Univariate and multivariable logistic regression analysis were used to determine independent predictive factors for deep involved LNM. Using logistic regression, odds ratios (OR) with 95% Confidence Interval (CI) were calculated for patient and melanoma characteristics in CLND and TLND patients.

Table 1. Patients and melanoma characteristics of all patients, CLND patients, and TLND patients

Characteristic		**All patients (n=129)**	**CLND (n=62)**	**TLND (n=67)**	**P**
Gender	Female	74	34	40	0.58
	Male	55	28	27	
Age	Median (range)	54 (22-89)	53 (23-88)	56 (22-89)	0.18
	<45	34	18	16	0.45
	45-54	32	18	14	
	55-65	34	13	21	
	>65	29	13	16	
Breslow thickness	Median (range)	2.23 (0.12-16)	2.95 (0.8-13)	2.10 (0.12-16)	0.10
	<1mm	10	2	8	**0.02**
	1-2mm	36	14	21	
	2-4mm	51	23	28	
	>4mm	25	18	7	
Ulceration	Absent	60	27	33	0.53
	Present	49	25	24	
Tumor mitotic rate	Median (range)	5 (0-23)	5 (0-20)	5 (0-23)	0.90
	Absent	7	2	5	0.57
	Present	86	34	52	
Clark level	III	17	6	11	0.47
	IV	89	41	48	
	V	12	7	5	
Location melanoma	Trunk	20	9	11	0.93
	Leg	102	47	55	
Histology	SSM	68	33	35	0.31
	NM	21	11	10	
	AL	7	1	6	
	Unknown	8	3	5	

CLND: Completion Lymph Node Dissection. TLND: Therapeutic Lymph Node Dissection. PM: Primary Melanoma. OR: Odds Ratio. CI: Confidence Interval. SSM: Superficial Spreading Melanoma. NM: Nodular Melanoma. AL: Acral Lentiginous.

Results

Characteristics of patients and their disease

A total of 129 patients were included in this study. The median age was 54 (range 22-89) years, while

74 patients (57%) were female and 55 (43%) were male. Median follow-up was 23 (range 0-177) months. In 62 patients CLND was performed, whereas in 67 patients TLND was performed. Further patient and melanoma characteristics are shown in Table 1.

Characteristics of lymph nodes

A total number of 2033 LNs were identified by means of histopatholgical analysis, with a mean of 16 (range 4-38) LNs per patient. From the superficial and deep areas of the groin a mean of 10.4 (range 2-26) and 5.3 (range 0-19) LNs were removed, respectively. Additional metastatic disease in the superficial LNs was found in 29 of the 62 CLND patients (47%). Metastatic disease was present in the deep LNs in 8 of 62 CLND patients (13%) and in 21 of 67 TLND patients (31%), which was a significant difference (P=0.012). This association was confirmed by logistic regression in all patients, the OR for having LNM in the deep LNs when TLND was performed was 3.61 (95%CI: 1.27-10.44, P=0.016) compared to CLND.

Table 2. Lymph node characteristics of all patients, CLND patients, and TLND patients

Characteristic		All patients (n=129)	CLND (n=62)	TLND (n=67)	P
Time from PM to LNM in months	Median (range)	4 (0-204)	1 (0-7)	50 (1-204)	
Number of removed LNs	Mean (range)	16 (4-38)	14 (4-37)	17 (5-38)	
Number of positive LNs	Mean (range)	3 (0-24)	2 (0-24)	4 (0-23)	
Number of superficial LNs removed	Mean (range)	10.4 (2-26)	9.1 (2-18)	11.5 (2-26)	
Number of deep LNs removed	Mean (range)	5.3 (0-19)	4.9 (0-19)	5.7 (0-17)	0.26
Positive superficial LNs	Mean (range)	3.09 (1-17)	3.31 (1-12)	2.56 (1-17)	
Positive deep LNs	Mean (range)	2.93 (1-12)	4.0 (1-12)	2.52 (1-6)	0.37
Patients with (additional) positive LNs in the LND	N (%)	95 (74%)	29 (47%)	67 (100%)	**<0.001**
Patients with positive superficial LNs	N (%)	92 (71%)	28 (45%)	66 (99%)*	**<0.001**
Patients with positive deep LNs	N (%)	29 (22%)	8 (13%)	21 (31%)	**0.012**
Size of LNs in cm	Mean (range)	2.9 (0.1-6.3)	2.8 (0.1-6.0)	3.0 (0.3-6.3)	0.49
Extracapsular growth	Absent	49	14	35	0.47
	Present	41	9	32	

* This patient only had a deep lymph node metastasis, no superficial lymph nodes were suspicious for malignancy. CLND: Completion Lymph Node Dissection. TLND: Therapeutic Lymph Node Dissection. LNs: Lymph Nodes.

The prediction of deep LNM

The only independent predictive factor for deep LNM in both CLND and TLND patients was the presence of more than 3 superficial LNM, predicting deep LNM with an OR of 20.00 (95%CI: 1.53-260.80, P=0.022) in CLND patients and 3.79 (95%CI:1.13-12.69, P=0.031) in TLND patients. In the univariate logistic regression, extranodal growth showed to be a predictor for deep LNM in TLND patients (OR:3.11, 95%CI:1.05-9.19, P=0.040), however, in the multivariable analysis this factor was not a significant predictor (OR:2.00, 95%CI:0.62-6.46, P=0.245).

Survival and recurrence analysis

Median MSS of CLND patients with only superficial LNM was 48 (range 0-177) months, while for CLND patients with both superficial and deep LNM it was 21 (range 2-55) months. The MSS of CLND patients with only superficial LNM was 74.0% after 2 years and 54.1% after 5 years. The 2-year survival for CLND patients with both superficial and deep LNM in the groin was 28.6%, while the 5-year survival was 14.3% for the same group. Survival was significantly worse for patients with deep LNM (HR:3.39, 95%CI:1.34-8.58, P=0.010) (Figure 1).

The median MSS for TLND patients with only superficial LNM was 34 (range 3-95) months, for TLND patients with involved deep LNs it was 22 (range 0-101) months. The 2-year MSS of TLND patients with only superficial LNM was 65.2% and the 5-year MSS was 37.2%, for TLND patients with both superficial and deep LNM this was 41.4% and 16.6%. MSS was significantly worse for patients with deep LNM (HR:2.01, 95%CI:1.04-3.88, P=0.039) (Figure 1).

The median DFP of CLND patients with only superficial LNM was 40 (range 0-174) months, for CLND patients with involved deep LNs it was 8 (range 0-18) months. The 2-year DFP of CLND patients with only superficial LNM in the groin was 62.8% and the 5-year DFP was 52.8%. A recurrence occurred in all CLND patients with deep LNM within 18 months, therefore the DFP was 0 for both 2 and 5 years. The median DFP of TLND patients with only superficial LNM was 25 (range 0-95) months, for TLND patients with involved deep LNs it was 15 (range 0-101) months. The DFP for TLND patients with only superficial LNM in the groin was 41.5% after 2 years and 22.9% after 5 years, and for TLND patients with superficial and deep LNM these values were 20.0% and 6.7%. Disease free period was significantly worse for CLND and TLND patients with deep LNM (HR:4.67, 95%CI:1.90-11.52, P=0.002 and HR:1.79, 95%CI:0.99-3.23, P=0.033) (Figure 2). The number of recurrences in the LNs of the groin and distant recurrences are presented in Table 2 for CLND and TLND patients with only superficial LNM or superficial and deep LNM. Distant recurrences were more common in CLND and TLND patients with deep LNM (75% and 59%) compared to CLND and TLND patients with only superficial LNM (13% and 46%). In the CLND group this was a significant difference (χ^2: 4.6, df 1, P=0.032) and the association between distant recurrences and the presence of deep LNM was confirmed with logistic regression (OR:5.53, 95%CI:1.02-30.10, P=0.048). In the TLND group this was not a significant difference.

Discussion

Incidence of deep lymph node metastases

The present study showed that incidence of metastases in the deep LNs of the groin was significantly lower in CLND patients (13%) than in TLND patients (31%). This is probably due to that TLND patients with macrometastases are already in a poorer stage of disease, and therefore the disease has already spread further in comparison with the CLND patients with micrometastases. Incidence of metastases in deep LNs in CLND and TLND patients ranges from 6-17% and 23-55% in the literature. [10,13,20-29] Therefore, considering this relatively high incidence of deep LNM it seems reasonable to perform a combined superficial & deep LND in both CLND and TLND patients.

Prediction of deep lymph node metastases in the groin

Ideally, superficial and deep LND is performed in one session. Therefore the presence of deep LNM in the groin should be preferably predicted before LND. However, the present study was unable to define patient or primary melanoma characteristics which predict the presence of deep LNM in the groin. Also other studies were not able to define histological or clinical factors to predict the presence of deep LNM. [8,25,26] Hence, it seems that the presence of deep LNM cannot be predicted using patient and primary melanoma factors.

Regarding SLN micrometastases, Zdzienicki et al. showed that none of the SLNB positive patients with micrometastases ≤1.0mm had deep LNMs. Therefore it seems safe in these patients to solely perform a superficial LND. [26] In our institution, SLNB positive patients with micrometastasis ≤1.0mm are already treated with a superficial LND and therefore these patients were not included in this study.

The present study showed that the presence of deep LNM could only be predicted by the number (>3) of superficial LNM. Other studies also described the number of involved superficial LNs to be an independent predictive factor for deep LNM. [24,26-31] A major disadvantage of the use of LN characteristics as predictive factor for deep LNM is that results of the superficial LND have to be awaited before deep LND can be performed. This means that superficial and deep LND cannot be performed in one session.

Imaging as a predictive factor for deep lymph node metastases

Van der ploeg et al. showed Computed Tomography (CT) can be used as a good predictor for deep LNM in TLND patients[13]. However, Allan et al. and Pasquali et al. did not show satisfactory results with CT for the prediction of deep LNM. [23,29] FDG-PET has been shown to have high accuracy in detecting distant melanoma metastases. [32] However, so far FDG-PET(/CT) has not been adequate in the detection of LNM. [33-35] The accuracy of detecting LNM is higher using lymphoscintigraphy combined with SPECT/CT (Single-Photon Emission Computed Tomography combined with CT), as SPECT/CT can identify deep LNM with a pattern of lymphatic drainage to obturator or iliac region. [36-38] However, none of the current imaging techniques can identify small (<3mm) LN or distant metastases. [38,39] Perhaps with the use of new tracers like [18F]ICF01006 the detection of smaller metastatic deposits with PET(/CT) could improve.[40]

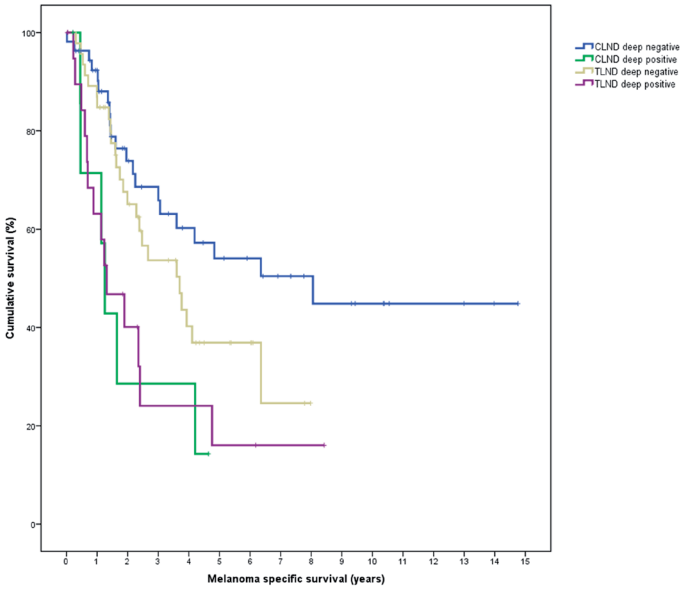

Figure 1. Melanoma specific survival of Completion and Therapeutic Lymph Node Dissection (CLND and TLND) patients with and without involvement of the deep lymph nodes

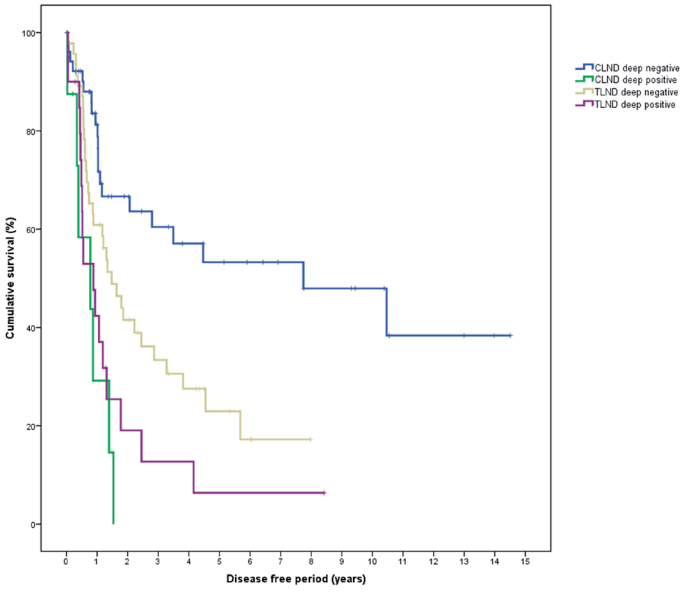

Figure 2. Disease free period of Completion and Therapeutic Lymph Node Dissection (CLND and TLND) patients with and without involvement of the deep lymph nodes

Table 3. Overview of studies describing 5-year overall survival rates of patients with and without deep lymph node metastases who underwent CLND or TLND

Reference	Year	CLND		TLND		Both CLND and TLND*	
		Superficial	Deep	Superficial	Deep	Superficial	Deep
Finck[41]	1982			38%	6%		
Coit[30]	1989			37%	6%		
Karakousis[42]	1994			43%	34%		
Karakousis[46]	1994			41%	28%		
Karakousis[43]	1996		50%		31%		
Mann[31]	1999			40%	35%		
Strobbe[45]	1999				24%		
Hughes[10]	2000			47%	19%		
Kretschmer[21]	2001				6%		
Meyer[22]	2002			36%	21%		
Badgwell[20]	2007					51%	42%
Nowecki[24]	2008	56%	28%	52%	36%		
Chu[28]	2011	70%	50%				
van der Ploeg[13]	2011			40%	12%		
Mozzillo[25]	2013					55%	32%
Zdzienicki [26]	2013					56%	33%
Current study	2013	54%	14%	37%	17%	46%	14%

* These studies did not separate patients in CLND or TLND groups. CLND: Completion Lymph Node Dissection. TLND: Therapeutic Lymph Node Dissection.

Survival and recurrence of patients with deep lymph node metastases

The present study showed that the survival CLND patients with only superficial LNM was significantly better compared to TLND patients with only superficial LNM, which is in concordance with other studies (Table 3).[13,21,22,24-26,28,30,31,41-44]The survival for CLND and TLND patients was significantly worse when the deep LNs were involved, which was also seen in other studies. [24,28,41,43] Interestingly, the survival of CLND and TLND was similar in CLND and TLND patients when the deep lymph nodes were involved. (14% vs 17%). Therefore the impact on prognosis of deep LNM in CLND patients is relatively worse compared to TLND patients. Moreover, the presence of deep LNM was associated with the presence of distant metastases in CLND patients and not in TLND patients. These results seem to indicate that melanoma patients with deep LNM have a melanoma with more aggressive tumor biology. In addition, the melanoma tumor biology seems to be relatively more aggressive in CLND patients with deep LNM compared to TLND patients with deep LNM. Another hypothesis is that the immune response of patients with deep LNM is poor and therefore metastatic melanoma cells can easily spread further

than the SLN. The poor survival and association with distant metastases is of clinical importance as with the presence of deep LNs a new group of patients can be defined with poor prognosis and this group might benefit from adjuvant systemic/radiation therapy.

Limitations and further research

This study was limited by its retrospective nature and by the relatively small number of patients, especially in the CLND group with deep LNM. Furthermore, imaging modalities were not taken into account for the prediction of LNM in the deep LNs. Randomized controlled trials are needed to determine whether routine superficial and deep CLND or TLND offers a survival benefit without compromising locoregional tumor control. Prospective studies MSLT-II[5] and EORTC 1208 (Minitub) [49]) will provide more information about which patients are better candidates for CLND and which for observation. Furthermore, the Australia and New Zealand Melanoma Trials Group (ANZMTG) are currently preparing for a trial that will compare overall survival, recurrence free survival, morbidity, and quality of life, between stage III melanoma patients undergoing superficial groin dissection vs combined superficial and deep groin dissection. [50] In addition, this trial will study the reliability of PET/CT for staging deep LNM.[50]

Finally, with new adjuvant systemic treatments, immunotherapy and/or drug targeted therapy, there is also a new opportunity to analyze the potential of performing only a superficial groin dissection, with adjuvant systemic treatment for patients with unfavourable tumor characteristics.Adjuvant systemic therapy is already performed in breast cancer[51] and will soon be analyzed for melanoma patients in the COMBI-AD trial, which will analyze the effect of adjuvant systemic treatment with a BRAF inhibitor combined with a MEK inhibitor on relapse-free survival in a placebo controlled trial for BRAF positive stage III melanoma patients. [52] Patients with locally advanced disease may be even candidates for a neoadjuvant approach with this new drug targeted inhibitors.[53]

Conclusions

The present study showed that deep LNM are fairly common in both CLND (13%) and TLND (31%) patients. The presence of deep LNM could not be predicted by patient or melanoma characteristics prior to performing the LND. The presence of deep LNM in the groin showed to be associated with poor prognosis in TLND patients and particularly in CLND patients. This poor prognosis of deep LNM could be a reason for future the use of adjuvant systemic and/or radiation therapy.

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Chapter 7

The prognostic significance of BRAF mutation status in stage IIIB-C melanoma

M.G. Niebling, K.P. Wevers, H.J. Hoekstra

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Abstract

Recent introduced systemic agents have shown promising results in melanoma patients with distant metastases. However, the durability of the effect of these agents is still disappointing. In melanoma patients with palpable regional lymph node metastases these new systemic drugs might induce longer efficacy as tumor load is low compared to patients with distant metastases. However, before administering these potentially toxic and still very expensive systemic drugs to these patients, selection of patients who are most likely to benefit from these drugs is important. In this key paper evaluation, the use of BRAF mutation status as a prognostic marker in stage III melanoma is discussed.

Introduction

In the study of Moreau et al[1], prognostic impact of the BRAF mutational status in stage IIIB-C melanoma patients was analyzed. They determined overall survival and distant metastasis free survival according to BRAF mutational status and adjusted for clinicopathologic factors in multivariable analysis. Moreau et al selected 145 stage III patients who underwent lymph node dissection between 2000 and 2010. To accurately assess BRAF mutational status only nodal deposits >2mm were selected for BRAF analysis leaving 105 patients. BRAF mutations were detected in 40% of these patients. Overall survival of patients with and without BRAF mutations were 16.7 and 39.7%, with a median overall survival of 1.4 and 2.8 years, respectively. Together with number of invaded lymph nodes (HR: 2.2, 95%CI: 1.3-3.9) BRAF mutation was an independent prognostic factor in the multivariable analysis (HR: 1.9, 95%CI: 1.2-3.1) for overall survival. Distant metastases occurred in 88.1 and 62% of the patients with and without the BRAF mutation with a median of 0.5 and 2.0 years to distant metastases, respectively. Multivariable analysis showed that the characteristics associated with distant metastases free survival were ulceration of primary melanoma (HR: 1.7, 95%CI: 1.0-2.8), the number of invaded lymph nodes (HR: 2.4, 95%CI: 1.3-4.3), and BRAF mutation (HR: 2.1, 95%CI: 1.3-3.4).

Discussion

Melanoma, an aggressive malignancy arising from melanocytes, now ranks as the sixth most frequently diagnosed cancer overall in western countries.[2] Due to the aggressiveness of the tumor approximately 16-28% of stage I or II patients develop recurrences.[2] The lymphatic route is a principal way of spread of melanoma cells and therefore these recurrences occur most often (26-60%) in the regional lymph nodes.[3] The regional control and survival of melanoma patients who develop clinically detectable nodal metastases (stage IIIB-C) is improved by performance of a therapeutic lymph node dissection.[4-9] Even despite accurate preoperative staging by PET/CT scanning a substantial proportion of patients develop distant disease shortly after therapeutic lymph node dissection, and 5-year survival rates remain unsatisfactory: 29-52%.[5,10]

Known prognostic factors in stage IIIB-C melanoma are number of invaded lymph nodes, nodal tumor load, lymph node ratio and extranodal growth.[4] Staging of patients with clinically detectable lymph node metastases is based on presence of ulceration of the primary tumor and number of invaded nodes in the 2009 classification guidelines of the American Joint Committee on Cancer (AJCC).[4] Radiation therapy, as an adjuvant treatment after lymph node dissection, can be considered to improve local control in patients with more than 3 nodes involved, nodes larger than 3cm, and/or extranodal growth pattern.[11]

Adjuvant systemic treatment with dacarbazine (DTIC) in stage III disease had no impact on survival.[12] High dose interferon-alpha 2b was approved for the adjuvant treatment in resected stage III disease in 1995. However, large randomized trials performed subsequently have not been able to reproduce

a benefit in overall survival.[13]

Recent studies have shown promising results using new systemic therapies in melanoma patients with distant metastases (stage IV): ipilimumab, an anti-CTLA4 antibody, and a combination of selective inhibitors of the BRAF or MEK signaling pathway.[14-16] Hodi et al showed an increase of median overall survival in patients receiving ipilimumab from 6.4 to 10.0 months.[16] Flaherty et al and Chapman et al showed promising results for BRAF inhibitors in stage IV melanoma patients.[15,17] Moreover, Flaherty et al recently showed that a combination of BRAF and MEK inhibitors compared to monotherapy with a BRAF inhibitor improved median progression-free survival from 5.8 to 9.4 months.[14]

Although the use of systemic treatments show promising response rates, side-effects of these drugs, especially those of ipilimumab, are serious. In addition, the durability of the anti tumor effect of BRAF and MEK inhibitors is still disappointing in these patients. Moreover, the combination of these inhibitors is only effective in roughly half of melanoma patients who have a BRAF mutation in their melanoma cells.

In stage III patients these new systemic therapies might induce longer efficacy as tumor load is low compared to stage IV patients, especially when lymph node dissection has already been performed. However, before administering these potentially toxic and still very expensive systemic drugs to stage III patients, selection of patients who are most likely to benefit from these drugs is important.

In theory, stage III patients with the worst prognosis will probably benefit most from adjuvant systemic treatment. Selection of these patients with poor prognosis can be partly based on clinicopathologic factors like those described in the AJCC staging manual. In addition, determination of serum biomarkers like S-100B, which is the most promising biomarker for predicting survival in melanoma, can be used to improve accuracy of selecting stage IIIB-C melanoma patients with worse prognosis.[18] Furthermore, this biomarker can be used in the stratification of new adjuvant trials.[18] Nevertheless, research is still being performed on prognostic factors in stage III patients in order to more accurately predict the prognosis of the individual patient.

The findings of Moreau et al[1] suggest that BRAF status is an important prognostic factor in stage IIIB-C patients, and that it contributes to a better estimation of the prognosis after lymph node dissection. Especially when BRAF status is combined with clinicopathologic factors and serum level of S-100B, a poor prognosis subgroup can be identified for adjuvant systemic therapy. Following the fact that BRAF mutations are predominantly seen in young patients, and this mutation enables therapy with BRAF and MEK inhibitors, this subgroup of patients seems ideal to select for this adjuvant systemic therapy.

Five year view

Interestingly, an upcoming trial (the 'COMBI-AD trial') will study the effect of adjuvant systemic treatment with a BRAF inhibitor combined with a MEK inhibitor on relapse-free survival in a placebo controlled trial. Patients eligible for this trial will be BRAF positive stage III patients who are treated by surgery with curative intent, even including sentinel node positive patients with lymph node metasta-

ses >1mm. The adjuvant use of BRAF inhibitors in this poor prognosis group, as shown by Moreau et al, is a promising setup. In five years we will have more information on this subject and perhaps adjuvant systemic treatment will play an important role in stage III melanoma patients. Nevertheless, results from the COMBI-AD trial have to be awaited to see if the benefits way up against costs and toxicity of this adjuvant systemic treatment in stage III melanoma patients.

Key issues

- The 5-year survival rates for stage III melanoma patients are still unsatisfactory: 29-52%
- Adjuvant treatment with dacarbazine or interferon-alpha 2b did not improve survival of stage III melanoma patients
- New systemic agents show promising results in stage IV melanoma patients
- Durability of the effect of these new agents is still relatively short in stage IV melanoma.
- Due to relatively low tumor load, efficacy of these new agents might be longer in stage III melanoma patients, especially in adjuvant setting.
- Selection of stage III melanoma patients, based on prognostic factors, could increase the potential of adjuvant systemic therapy, and reduces unnecessary morbidity and costs.
- Moreau et al [1] showed the BRAF status to be a prognostic marker in stage IIIB-C melanoma patients, meaning that besides a therapeutic target BRAF can also be used to define a group of patients with poor prognosis.
- The upcoming COMBI-AD trial will investigate the use of BRAF inhibitors in stage III melanoma patients. The results of this trial will guide the future use of adjuvant systemic therapy in these patients.

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Part III

Imaging modalities in melanoma

Chapter 8

Melanoma, imaging

M.G. Niebling, E. Bastiaannet, O.S. Hoekstra, H.J. Hoekstra

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Abstract

Accurate diagnosis at an early clinical stage is one of the most important factors for successful management in melanoma, allowing treatment to be undertaken when cure is still achievable. Treatment of melanoma is guided by primary melanoma characteristics and the possible presence of lymph node or distant metastases. Consequently, accurate staging is extremely important for clinical decision making to identify those patients who may benefit from surgery while avoiding unnecessary, potentially harmful surgery that does not improve survival. Furthermore, accurate staging is important to properly select patients for tri als and patient counseling on prognosis. Currently the biomarker S-100B is often used for this purpose; it is the most promising melanoma biomarker in predicting survival in melanoma patients. Imaging with computed tomography (CT), magnetic resonance imaging (MRI) and especially molecular imaging play an important role in staging of melanoma patients.

Sentinel lymph nodes are currently detected with the use of sentinel lymph node biopsy; this procedure is assisted by preoperative lymphoscintigraphy with ^{99m}Technetium as a radiotracer for localizing sentinel lymph nodes. For the detection of distant metastases a FDG-PET is used. This radiopharmaceutical is very effective for localizing melanoma cells as these are typically FDG-avid. However, uptake of FDG is also seen in inflammation, infection, and is also taken up by muscles and the central nervous system. Furthermore, FDG-PET sensitivity is lower in detecting melanoma foci in lung, liver and brain. New radiopharmaceuticals specific for melanomas may offer better capacity for further PET diagnostics. In stage I and II, and microscopic stage III patients FDG-PET(/CT) has no additional value. However, for patients with palpable, proven lymph node metastases with no suspicion for lung metastases on X-ray, FDG-PET(/CT) does have additional value in treatment planning. Also, for stage IV melanoma patients FDG-PET(/CT) may be of importance to localize the distant metastases if surgical treatment is considered. MRI of the brain is the procedure of choice in melanoma patients with symptoms related to the CNS. It is a mandatory test in stage IV melanoma patients, optional in stage III melanoma patients and not recommended in patients with stage I and II melanoma.

Finally there are current vibrant developments of optical imaging systems for intraoperative fluorescence epi-illumination.[61] This technology enhanced surgical vision of metastases in the operating room and during surgery. Optical imaging will not replace the previous described modalities but is an additional tool for improving staging and treatment of melanoma in the near future.

Synopsis

Treatment of melanoma is guided by primary melanoma characteristics and the possible presence of lymph node or distant metastases. Accurate diagnosis is one of the most important factors for successful management in melanoma, allowing treatment to be undertaken when cure is still achievable. For the detection of sentinel lymph nodes or distant metastases nuclear imaging modalities can be used. For the detection of distant metastases in melanoma patients positron emission tomography (PET) with Fluorodeoxyglucose (FDG) is most frequently used, which is very effective as melanoma cells are typically FDG-avid. This chapter discusses the value of imaging modalities with a special focus on FDG-PET(/CT) in staging and follow-up of melanoma patients.

Study protocols

Protocol FDG-PET/CT:

A summary of the FDG-PET/CT protocol is stated below, the full guideline for this protocol was published by Boellaard et al[1] and is stated in 'Further reading'

Preparation and execution of FDG-PET/CT:

- In case of manual administration:
    - An indwelling intravenous device is used to administer the FDG intravenously once the patient's blood glucose has been determined and blood samples for laboratory testing have been taken if necessary. Make sure that if there is a needle on the syringe it is free from FDG.
    - Flush and rinse out the administration syringe with at least 10 ml of normal saline (NaCl 0.9%) using the three-way valve.
- In case of automated administration:
    - Make sure that the automated system and procedures assures a net administered FDG activity within 3% accuracy (this must be ensured by manufacturer and verified by the user), i.e. the actual administered activity may not deviate more than 3% from that indicated by the reading of that device or used dose calibrator. Follow instructions given by the manufacturer.
- The administration system can be removed after intravenous administration (unless CT contrast agent is to be administered subsequently by intravenous injection).
- The ambient conditions in the waiting room must be relaxing and warm. Give the patient extra blankets if necessary.
- Tell the patients to lie or sit as calmly as they can, and not to talk. Provide comfortable beds or chairs. They may go to the toilet while waiting, preferably after the first 30 min p.i. Ask the patient to use the bathroom 5 min before the start of the PET study.
- An intense bladder or ureter activity concentration can impair the interpretation of lesions in the

pelvis and retroperitonium. Hydration and loop diuretics (e.g. furosemide intravenously) may be used to reduce bladder activity and radiation exposure to the bladder. Therefore, during the waiting period, patients will be asked to drink another half a litre of water, or this amount can be given in the form of physiological saline intravenously, if such fluid load is not medically contra-indicated. This is of course dependent on the patients other clinical conditions, e.g. impaired renal function or poor cardiac function, where this amount of fluid may be contraindicated.

- The recommended interval between FDG administration and the start of acquisition is 60 min. However, for certain clinical trials this may change depending on the disease and aims of the study. The actual interval should be recorded, i.e. the time of FDG injection (administration) should be reported. When repeating a scan on the same patient, especially in the context of therapy response assessment, it is essential to apply the same interval (tolerance ±5 min). In addition, use of the same PET or PET/CT system and identical acquisition and reconstruction settings must be applied when making multiple scans of the same patient.
- Scan trajectory: for most oncology indications, a wholebody scan is sufficient. A 'whole-body' uptake normally covers the part of the body from the mid-femora to the external auditory meatus (in that direction, as bladder activity increases during the scan). A longer scanning trajectory may be used if appropriate: for melanoma, many institutions apply a total body examination routinely, or depending on the site of the primary tumor.
- The patient should be positioned with their arms elevated over the head to avoid beam hardening artifacts as well as artifacts caused by truncation of the field of view. For the examination of head and neck tumors, a two step protocol is recommended (head and neck portion and from the apex of the lung through mid thigh) with the appropriate acquisition and reconstruction parameters adapted for the protocol. Alternatively, the arms can be positioned along the side for head and neck imaging. If the FDG PET/CT data are used for radiation planning, the examination should be carried out in the radiation position using the same dedicated radioopaque positioning devices as used in the radiotherapy department (e.g. same table tops, laser alignment, immobilisation measures, etc.).
- Scan acquisition depends on various factors, including the system type and acquisition mode (2D, 3D). For CT settings in case of PET/CT, CT whole-body or low-dose CT, see Other acquisition parameters, CT-protocol. Transmission scanning time for each bed position depends on whether the scan is a CT scan or a transmission scan with Ge-68/Ga-68 source.
- In general, PET/CT is carried out using a protocol comprising a scanogram/scout scan/topogram and a low-dose CT for attenuation correction (CT-AC) and anatomical correlation. IV contrast agent must not be administered during the low-dose CT, used for attenuation correction purposes, because of its potential influence on Standardized Uptake Value (SUV; see below) calculation.
- In the case of single slice or dual-slice CT, artifacts are created in the diaphragm area when

the patient breathes. The patient must therefore hold his/her breath for a few seconds on the technician's instructions during CT-AC acquisitions. No such instructions need be given in the case of PET/CT systems with more than two slices. The CT-AC scan can then be carried out while the patient continues to breath shallowly.

- A standard diagnostic CT scan with (intravenous) contrast agent may, if appropriate, be carried out according to standard radiological methods after the low-dose CT and PET acquisition in case quantification of the PET study will be performed or is required.
- Recommendations for FDG activities are based on assuming a fixed scan duration of 5 min per bed position and a bed overlap of less than 25%. In the case of 2D scans: ca. 5 MBq/kg body weight (±10%). In the case of 3D scans: ca. 2.5 MBq/kg body weight (±10%).

Further reading

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Introduction

According to estimates for 2008, there were almost 200,000 new cases of invasive cutaneous melanoma, and an estimated 46,000 deaths from this disease.[2] The vast majority of cases (almost 85%) occur in developed countries, where melanoma ranks sixth of the most frequently diagnosed cancers.[2] The incidence of melanoma has increased dramatically in Caucasian populations in all parts of the world; it is one of the tumors with the most rapidly increasing incidence among all malignancies.[3-7]

The most important factor for successful management of melanoma is early diagnosis, allowing treatment to be undertaken at a stage when cure is still achievable.[8] Probably due to increased awareness in the general population, melanoma is now more diagnosed at an earlier stage. The median Breslow thickness at first clinical presentation declined over time and the majority of patients are diagnosed with stage I or II melanoma.[9] However, approximately 16-28% of these patients develop recurrences; locally or in transit in 20–28%, distant in 15–50%, but most frequently in regional lymph nodes (26–60%).[10]

Wide local excision with proper resection margins according to the thickness of the lesion remains the

treatment for stage I or II melanoma. The 10-year survival rates for stage I are 85-97% and for stage II 39-68%.[11] Prognostic factors for stage I and II melanoma are gender, Breslow thickness, ulceration, tumor mitotic rate, and localization of the tumor.[11] The survival is worse for melanoma patients with metastases in the lymph nodes (stage III), the 5-year survival ranged from 40-78%.[11] Prognostic factors within stage III melanoma are number, size, and extranodal growth of the lymph node metastases.[11] The lymphatic route is a principal way of spread of melanomas from their original focus, the melanoma cells progressing via the lymphatic vessels are stopped in the first node on the way: the sentinel lymph node. The interim results of MSLT I trial (randomized controlled trial that compared observation of regional lymph nodes with only lymphadenectomy if nodal relapse occurred with sentinel-node biopsy with immediate lymphadenectomy if nodal micrometastases were detected on biopsy) showed that sentinel lymph node biopsy (SLNB) provides important prognostic information and identifies patients with nodal metastases whose survival can be prolonged by immediate lymphadenectomy[12] (the final analyses of the MSLT I Trial will be published in 2013). This SLNB procedure has become widely accepted and is today recommended by the American Society of Clinical Oncology (ASCO) and Society of Surgical Oncology (SSO) in patients with melanoma ≥1mm for accurate staging and part of the American Joint Committee on Cancer (AJCC) tumor staging.[10,11]

Patients with distant metastases (stage IV) have a worse prognosis, the most recent drug targeting trial with BRAF kinase and mitogen-activated protein kinase (MAPK) inhibitors showing a median progression free survival of 9.4 months.[13] Prognostic factors for stage IV melanomas are localization and number of distant metastases, and probably serum levels of S-100B.[11,14-16] Indeed, in patients with only a few and resectable distant metastases, surgical resection seems to have a survival benefit (median survival of 22 months).[14] As a consequence, the major objective in the treatment planning of high risk (stage III) melanoma patients is the earliest possible detection of metastases. However, melanoma has the well-known feature to spread to unusual sites, and multiple distant metastases are usually a contraindication for surgery.[17] Imaging with computed tomography (CT), magnetic resonance imaging (MRI) and especially molecular imaging play an important role in staging of melanoma patients. Fluorodeoxyglucose (FDG) positron emission tomography (PET) can be a valuable tool to screen for metastases since melanoma typically is very FDG avid and since a whole body scan covers the full, often erratic pattern of spread (except for areas with high physiological FDG uptake like the brain). This chapter will discuss the value of imaging modalities with a special focus of FDG-PET(/CT) in staging and follow-up of melanoma patients.

Radiopharmaceuticals

Detection of sentinel lymph nodes

The presence or absence of regional lymph node metastases is an important prognostic factor for patients with melanoma. SLNB using a combination of a radiotracer and blue dye to identify the sentinel lymph nodes and has proven accuracy in nodal staging.[18] FDG-PET has limited sensitivity to detect

microscopic lymph node metastases.[19,20] Sensitivity and specificity rate of FDG-PET for sentinel nodes is 17% and 92%, respectively.[21] The poor sensitivity can be explained by that FDG-PET, in contrast with SLNB, is unable to detect micrometastatic disease. Havenga et al found metastases in regional nodes in 13 of 53 patients with SLNB, FDG-PET was positive in only 2 of these 13 patients.[19] Moreover, in a study of Acland et al FDG-PET did not identify metastatic disease in the sentinel lymph node or draining basin in any of 14 patients where SLNB was positive.[22] Although the accuracy of FDG-PET to detect lymph node metastases is higher in patients with clinically palpable lymph nodes, the clinical relevance of these findings is limited since fine needle aspiration (FNA) biopsies are quite effective to solve the clinical problem.[23] Biopsy of the sentinel lymph node offers a highly sensitive and specific staging method.[21] Sentinel lymph node biopsy (SLNB) is performed using a combination of a radiotracer and blue dye to identify the sentinel lymph nodes. The colloid compounds used at present (albumin, sulphur-, tin-, antimonium trisulphide colloid, and albumin nanocolloid) are labeled with ^{99m}Tc, a metastable nuclear isomer of technetium-99. After intracutaneous peritumoral injection of a radiocolloid, the drainage pattern to the regional lymph nodes is dynamically monitored using a gamma camera, and, within approximately 24 hours, the first-draining (sentinel) nodes are harvested guiding the surgeon using a hand-held gammaprobe. The combination of this method with the use of blue dye (as a visual aid to the gammaprobe signal) has a typical detection rate of 94.5%.[21]

Detection of distant metastases

FDG-PET

At present, FDG-PET imaging of melanoma is the most common PET clinical radiotracer used routinely to localize melanoma. In a recent study accuracy, sensitivity, and specificity for FDG-PET were 91% (95% confidence interval (CI): 87%-95%), 86% (95%CI: 78%-94%), and 93.1% (95CI: 89%-97%), respectively, for detection of distant metastases in clinically stage III melanoma patients.[24] Although FDG is an effective tool for melanoma detection, inflammation and infection compromise its specificity, and partial volume-effects limit its sensitivity for small-volume disease. Moreover, the biodistribution of FDG implies several areas with very limited detection possibilities (most importantly, the brain).[25] Therefore, other radiopharmaceuticals have been investigated (see below).

Quantification of the FDG signal

Standardized uptake value (SUV), which represents the FDG accumulation in the tumor or metastasis normalized for the injected dose and the volume of FDG distribution, could be a useful index in different cancers. It could be used to predict prognosis, as metabolic activity correlates with tumor proliferation and therefore with biologic aggressiveness.[26] Although there is ample proof of principle that FDG PET may add value to anatomy-based response, the clinical usefulness and applicability of SUV for prognostic purposes is still under discussion.[27,28] Even though several studies showed no significant association between SUV and overall survival in stage III melanoma patients,[27-29] some reported an

inverse association between disease free survival and SUV.[27,28]
It should be emphasized that quantitative PET measures as biomarkers of prognosis or response can only be validated and implemented if strict procedures are maintained for scanner calibration, and during PET acquisition, image reconstruction and analysis since each may have profound effects on the results.[1] Furthermore, partial volume effects (underestimating SUV in lesions < 2 cm with current post-image reconstruction resolution) can be strong confounders in prognostic or predictive research. In the past decades, the consequences of quality assurance and quality control have been underestimated so that meta-analysis (essential for biomarker validation) is very difficult.[30]

Response assessment with FDG-PET
Heterogeneity of FDG-PET radiopharmaceutical uptake has been shown to reflect differences in tumor biology and may be predictive for response in treatment. In breast cancer for example, 48% of patients exhibited a heterogenous FDG-PET response and heterogeneity correlated with time to progression.[31] In stage IV melanoma, two studies showed that SUV is rapidly and homogenously reduced after starting systemic treatment with BRAF inhibitors[26,32] If these observations are confirmed in larger studies, SUV might be used for predicting tumor response for these drugs. Since FDG-PET is already performed in staging melanoma, determination of SUV values would provide additional information at very little extra cost.[28]

Radiopharmaceuticals with specific affinity to melanoma cells
Radioimmunoscintigraphy
Radioimmunoscintigraphy utilizes radiolabelled monoclonal antibodies (mAB) or their fragments (Fab) with a specific affinity to antigens present on neoplastic cells. A study showed sensitivity and specificity rates of 79% and 100% with fragment Fab2 of the antibody 255-28 S for detection of ocular melanoma.[33] However, wider use of radioimmunoscintigraphy in diagnostics is hampered by the costs of preparation and by the fact that monoclonal antibodies are large molecules which migrate slowly in the tissues, leading to high background after introduction of this radiopharmaceutical into the system, especially ^{99m}Tc labeled mAB as they have a relatively short half life. In radioimmunoscintigraphy it is important to use isotopes which half life is as close as possible to the half life of the molecule that is studied. ^{111}Indium labeled mAB showed to have longer half life, however further research is needed to test the potential of this radiopharmaceutical.[25]

Melanotropin analogues (alpha-MSH)
Melanoma cells have high affinity to melanotropic hormone (alpha-melanocyte stimulating hormone). The hormone stimulating melanocytes act on the cell via the MelanoCortin type 1 Receptor (MC1R). MC1R is a G-protein coupled receptor which is over-expressed in many types of melanoma, making it an attractive target for receptor based melanoma imaging and therapy. Peptide based radioactive probes

have been extensively studied for tumor receptor targeted imaging and therapy. With 18F labeled α-MSH as a radiopharmaceutical a-specific uptake prevailed in lung, liver and gallbladder.[34] However, recent research showed promising results of 18F labeled α-MSH metallopeptides in vivo, with less lung and liver uptake compared to previous studies.[35] Nevertheless, further studies are required for further analysis of this peptide.

Iodinated aminoalkyl benzamide derivatives

Several studies have been performed to analyze the use of numerous compounds from the group of N-alkylated benzamide derivates labeled with radioactive nuclides (^{123}I, ^{131}I) in the diagnostics of melanoma. Although long uptake interval time (18-24h) and accumulation of the compound in the liver were drawbacks for benzamide derivates, one study showed sensitivity and specificity of 81% and 100%, respectively, for detection of distant metastases in patients with malignant melanoma.[36] Moreover, recent studies showed selective uptake with high tumor/non-tumor ratios of benzamide derivates and shorter uptake interval time (1-2h).[37,38] In an in vitro study the tumor-to-background ratio of benzamide ^{18}F-MEL050 was more than 9-fold higher compared to ^{18}F-FDG in a melanoma allograft model.[37,38] This provides optimism for applying these derivates as a radiopharmaceutical for melanoma.

Conclusions

Molecular imaging techniques are being improved to overcome specificity issues with FDG, and to enhance signal-to-background ratios by improving targeting and/or capitalizing on lower background tracer uptake. Lymphoscintigraphy with ^{99m}Technetium-colloids to identify sentinel lymph nodes and PET with FDG to detect distant metastases are currently standard in clinical practice. Numerous other radiolabeled compounds are being investigated, and may be promising especially in combination with PET. Further improvement might be achieved by combining molecular, functional and anatomical imaging using PET-MRI. FDG-PET can also be used for determining prognosis or systemic therapy response with SUV.

Technical considerations

Imaging modalities in early (stage I and II) melanoma

As discussed in the preceding section accurate nodal staging by detection of sentinel lymph nodes is important in melanoma staging.[11] FDG-PET also showed disappointing results in detection of distant metastases in stage I and II melanoma with FDG-PET. A recent review showed FDG-PET to detect metastatic disease of melanoma in 38 of 609 stage I and II melanoma patients, but only one of these patients (0.16% of 609) was subsequently found to have metastatic disease.[21] Moreover, 10 patients were found to have other neoplastic processes and in 27 of 609 patients a false positive result was found. Not unexpectedly, and as with any low prevalence condition, these results substantiate the

intuitive notion that FDG-PET does not add significant information in staging of early melanoma and that it should not be routinely used in such patients.

Sentinel node biopsy: lymphoscintigraphy and SPECT/CT in SLNB

Lymphoscintigraphy is often performed preoperatively, this imaging technique has proven to be of use for sentinel lymph node mapping.[21] However, single-photon emission computed tomography/computed tomography (SPECT/CT) has shown important benefits compared to planar lymphoscintigraphy in sentinel lymph node mapping.[39] With conventional planar imaging an interval sentinel node may be difficult to distinguish from lymphangioma, lymphatic lake, or skin contamination. Images of SPECT/CT combine physiologic and morphologic properties, resulting in an enhanced sentinel node identification because of superior contrast and resolution that can resolve such dilemmas.[39] A recent study reported that SPECT/CT provided additional sentinel lymph nodes or additional anatomic information used for surgical planning in 16 (46%) of 35 patients compared to conventional lymphoscintigraphy.[40] The three-dimensional reconstruction images are a helpful tool, providing a simple yet comprehensive overview of the localization of hot spots. This type of image fusion provides better anatomical benchmarks, provides schematic information about the sentinel node site, and (perhaps most importantly) is easy to understand for surgeons, medical staff, and patients.[39] Nevertheless, conventional lymphoscintigraphy currently remains the only technique available to visualize the dynamic process of lymphatic drainage. Therefore SPECT/CT does not replace the conventional planar images; it can be considered as a complementary modality.

Imaging modalities in advanced (stage III and IV) and recurrent melanoma

In advanced and recurrent melanoma there is greater likelihood of distant metastatic disease. Accurate staging is of great importance in these patients. Firstly, to identify those patients who may benefit from a surgical procedure, while avoiding these potentially harmful surgical procedures for patients with multiple distant disease.[23] Patients with only lymph node metastases can be cured with a therapeutic lymph node dissection (TLND). Patients with distant metastases could benefit from surgery, targeted therapy and/or immunotherapy. Staging with FDG-PET/CT is important in these patients to determine if surgical resection is feasible as this increases 5-year survival of stage IV melanoma patients to 40%.[14] Secondly, accurate staging with FDG-PET/CT is important to improve efficacy of clinical trials. Thirdly, accurate staging is important to provide patients with accurate information about their prognosis.[23]

A review of studies which analyzed their data on a per patient/scan basis showed high accuracy (88%, 95%CI: 86%-90%), sensitivity (86%, 95%CI: 83%-89%) and specificity rates (89%, 95%CI: 86%-91%) for FDG-PET in detecting distant metastases in melanoma patients.[21] Two studies showed that FDG-PET is of additional value in clinical stage III patients.[41,42] Sensitivity rates were high (87% and 86%) for distant metastases in these patients and in the first study 27% of the patients were upstaged to stage IV. FDG-PET has shown to be particularly accurate in specific anatomical sites: soft tissue metastases,

intra-abdominal foci, and bone metastases.[21] FDG-PET is less accurate in liver metastases, pulmonary metastases, and cerebral metastases, although this can be overcome, especially in liver and lung, with the use of the combined FDG-PET/CT, which is currently standard in most medical centers in developed countries. Images of FDG-PET and FDG-PET/CT of a melanoma patient with distant metastases are shown in figure 1 and 2. Meta-analyses performed to examine the utility of FDG-PET/CT, FDG-PET alone, and CT alone for the staging and surveillance of patients with melanoma based on 10,528 patients between 1990 and 2009 found FDG-PET/CT to be the most accurate modality for the detection of distant metastases.[43] However, it has been noted that FDG-PET/CT can also miss small lesions in lung and liver. Currently for accurate estimation of prognosis in stage III melanoma patients determination of serum biomarkers like S-100B can also be used to improve accuracy for stage IIIB-C melanoma patients. Furthermore, this biomarker can be used in the stratification of new adjuvant trials.[16]
FDG-PET/CT does not seem useful in the follow-up of melanoma patients. Not many studies have been performed on the use of FDG-PET/CT in follow-up. This is partly due to the fact that recurrences are (usually) detected by the melanoma patients themselves. Moreover, the most essential component of surveillance to detect recurrences or new primary melanomas is the history and physical examination. One study showed FDG-PET/CT to be a valuable modality in 20 clinical stage III melanoma patients. Further research with a larger study population, taking cost-effectiveness as well as total radiation into account, is required before FDG-PET/CT can be justified in the follow-up of melanoma patients.[44]

Change in management due to FDG-PET(/CT)

Results of the FDG-PET(/CT) can lead to a change in management of melanoma patients; studies range from 17% to 49% with FDG-PET in stage I to IV patients.[41,45-52] A study in exclusively stage III patients found a change in management of 19%.[41] Change in management due to FDG-PET/CT occurred in 12%, 48.4%, and 57.6% according to three other recent studies.[53-55] The second study analyzed stage I to IV melanoma patients,[54] whereas in the first and third study stage III and IV (oligometastatic stage IV) melanoma patients were analyzed.[53,55]
Although FDG-PET and CT are expensive imaging modalities, adding FDG-PET and/or CT to the diagnostic work-up of clinically stage III melanoma patients does not increase costs substantially.[24] The cost-effectiveness of combined FDG-PET/CT is yet to be studied.
Thus, FDG-PET/CT appears to be an accurate staging method in advanced and recurrent melanoma. However, false-positive findings can occur, these include inflammation such as postoperative wound infection, pneumonia, pseudolymphoma, reactive changes within a lymph node, inflammation in an epidermal cyst and endometriosis.[21] Therefore, if FDG-PET/CT imaging indicates a radical change in management an attempt to confirm the FDG-PET/CT findings (by biopsy or other imaging) is required.

Brain imaging in melanoma patients

The high physiological uptake of FDG in the normal brain limits the sensitivity for detecting brain

metastasis, which is a frequent metastasis site in patients with melanoma.[56] Magnetic resonance imaging scan (MRI) is the current gold standard for this purpose.[57] The necessity of performing routine brain imaging in asymptomatic patients with advanced locoregional disease is controversial. Some clinicians perform the procedure only in symptomatic patients to rule out central nervous system involvement, while others recommend brain imaging before definitive local therapy in accordance with a report showing a rate of asymptomatic central nervous system metastases as high as 6%.[58] MRI of the brain in asymptomatic stage I and II melanoma patients shows low detection rates and (as to be expected) a high frequency of false-positives and is therefore not recommended in these patients. Although early detection of brain metastases may identify a limited number of patients who are eligible for more aggressive local therapies, no available data demonstrate that screening for brain metastases results in a survival benefit for patients. A recent study showed that in only 1.6% of stage III melanoma patients MRI detected lesions suspicious for melanoma metastases.[56] Furthermore, two other recent studies showed no brain metastases detected by MRI in stage III melanoma patients.[59,60] Therefore routine MRI of the brain does not seem advisable for stage III melanoma patients. Stage IV melanoma patients should be evaluated with MRI of the brain because the likelihood of detecting additional asymptomatic lesions is high and management of stage IV patients can change due to the detection of brain metastases in these patients.

Guideline and recommendations for the use of FDG-PET(/CT) in melanoma

According to recent research FDG-PET or FDG-PET/CT seem valuable added to the diagnostic work-up of clinical stage III and stage IV melanoma patients.[17,21,41-43,45-55] Moreover, adding FDG-PET and/or CT does not increase costs substantially in stage III melanoma patients.[24] In stage I and II, and SNB positive patients FDG-PET(/CT) has no additional value. However, for patients with palpable, proven lymph node metastases with no suspicion for lung metastases on X-ray, FDG-PET/CT does have additional value vs. CT alone in terms of treatment planning. Also, for stage IV melanoma patients FDG-PET(/CT) may be of importance to localize distant metastases if surgical treatment is considered. Verification of FDG-PET(/CT) findings remains necessary in patients to prevent that potentially beneficial surgery for localized disease is withheld.

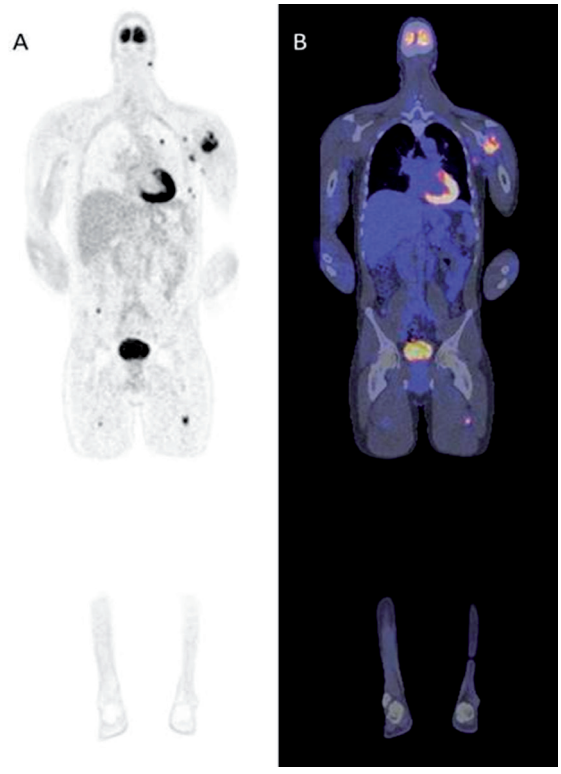

Figure 1. Images of FDG-PET (A) and FDG-PET/CT (B) of a melanoma patient with distant metastases in lung, bone, muscle, and subcutaneous tissue

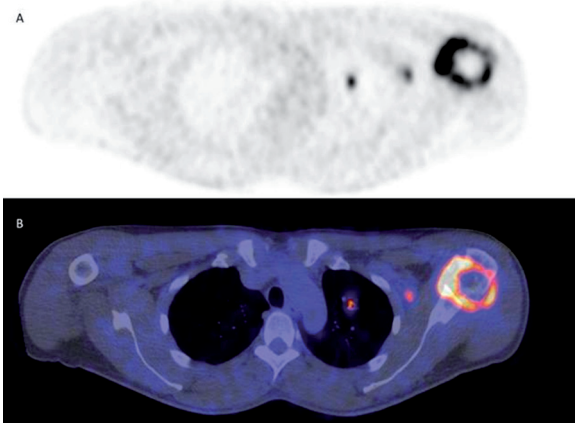

Figure 2. Images of FDG-PET (A) and FDG-PET/CT (B) of a melanoma patient with distant metastases in lung, bone, and subcutaneous tissue

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Chapter 9

Outcome of clinical stage III melanoma patients with FDG-PET and whole body CT added to the diagnostic work-up

M.G. Niebling, E. Bastiaannet, O.S. Hoekstra, J.J. Bonenkamp, R. Koelemij, H.J. Hoekstra

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Abstract

Background

Combined whole body FDG-PET and CT provide the most comprehensive staging of melanoma patients with palpable Lymph Node Metastases (LNM). The aim of this study is to analyze survival of FDG-PET and CT negative or positive melanoma patients and to assess which factors have independent prognostic impact on survival of these patients.

Methods

Patients with palpable and histologically or cytologically proven LNM of melanoma, referred to participating hospitals for examination with FDG-PET and CT were selected from a previous study. Melanoma Specific Survival (MSS) and Disease Free Period (DFP) were analyzed for FDG-PET and CT positive and negative patients using the Kaplan Meier method. Cox-regression analysis was performed to analyze which patient or melanoma characteristics had significant impact on MSS or DFP.

Results

For all 252 patients 5-year MSS was 38.2%. For FDG-PET and CT negative and positive patients 5-year MSS was 47.6% and 16.9%, respectively. Disease free period for FDG-PET and CT negative patients was 46.0% after 5 years. Gender, a positive FDG-PET and CT, LNM in axilla compared to head or neck, and presence of extranodal growth were independent factors for worse MSS in all patients. Positive FDG-PET and CT was the most important prognostic factor for MSS with a Hazard Ratio of 2.54 (95%-CI: 1.55-4.17, P<0.001).

Conclusion

Staging melanoma patients with palpable LNM is more accurate when whole body FDG-PET and CT is added to the diagnostic work-up. Hence, FDG-PET and CT, preferably combined, are indicated in the staging of clinical stage III melanoma patients.

Introduction

Incidence rates of melanoma are rising in the Netherlands, especially in the elderly.[1,2] In the last decade the incidence increased from 15.5 per 100 000 in 2000 to 28.1 per 100 000 in 2010.[1,2] Although Breslow thickness declined over time and the majority of the diagnosed melanomas are initially stage I or II,[2] approximately 16-28% of these patients develop recurrences; locally or in transit in 20–28%, distant in 15–50%, but most frequently in regional lymph nodes (26–60%).[3]

Regional Lymph Node Metastases (LNM) are nowadays often detected by sentinel lymph node biopsy (SLNB), which is a procedure recommended by the American Joint Committee on Cancer (AJCC).[4] This procedure has become widely accepted and is also recommended by the American Society of Clinical Oncology (ASCO) and Society of Surgical Oncology (SSO) in patients with melanoma ≥1mm for accurate staging.[5] However, regional LNM are also often detected clinically, usually detected by the patients themselves.[3] To achieve regional disease control, therapeutic lymph node dissection (TLND) is recommended in patients with palpable lymph nodes.[5] After TLND for palpable LNM, 5-year survival rates range from 26-43% and disease free 5-year survival ranges from 19-27%[4,6-9] Of all recurrences after TLND, 26-49% were locoregional and 51-74% were distant metastasis recurrences.[9,10]

Patients with distant recurrences (AJCC stage IV) have the worst prognosis with 5-year survival rates ranging from 4.9-11%.[11-14] The location and number of metastases are factors which influence survival of stage IV melanoma patients.[4,15,16]

The present study was designed to analyze the follow-up of [18F]fluorodeoxyglucose (FDG) positron emission tomography (PET) and computed tomography (CT) staged patients from the study of Bastiaannet et al, which was designed to compare FDG-PET with CT in melanoma patients with clinically palpable lymph nodes.[17] The primary aim of the present study is to analyze the follow-up of both the FDG-PET and CT negative patients and positive patients.

Methods

Patients

Patients with palpable, histology or cytology proven LNM (not sentinel node staged) of melanoma were selected from the study of Bastiaannet et al that compared FDG-PET and CT in staging melanoma patients.[17] These patients were referred to five participating hospitals (July 2003 through December 2007) for examination with FDG-PET and CT, the study was approved by the medical ethical committees of all participating hospitals.

A total of 252 patients with palpable, histologically or cytologically proven melanoma LNM underwent a FDG-PET and CT-scan to identify distant metastases (details are described in Bastiaannet et al).[17] Depending on the result of the FDG-PET and CT-scan patients were classified, using the AJCC melanoma staging guidelines of 2009,[4] as stage III (b or c) (n=173) or as stage IV (n=79).

Data analysis

Medical records of the patients were searched to collect the following data: age; gender; date and localization of the primary melanoma and LNM; Breslow thickness (mm); tumor mitotic rate (TMR) measured in mitoses per mm^2; ulceration; Clark level of invasion (in category I-V); number of removed and positive nodes during TLND; size of largest lymph node (cm); absence/presence of extranodal growth; results FDG-PET (positive/negative for distant metastases); results of CT (positive/negative for distant metastases); location of distant metastasis; date and first site (local, regional or distant) of the recurrence and survival data.

MSS with death due to melanoma as event for all patients, and FDG-PET and CT negative and positive melanoma patients was calculated and DFP with recurrence as event was calculated only for FDG-PET and CT negative patients, also both loco-regional free period and distant metastases free period were calculated for these patients.

Statistical analysis

The Chi square test was used to calculate if there was a significant difference to a level of $p<0.05$ in categorical variables of FDG-PET and CT negative and positive melanoma patients. For continuous variables Mann Whitney-U test and the Independent T-test were used, depending on the distribution of the variable. Survival curves for MSS and DFP were constructed by the Kaplan Meier-method and differences were assessed using the log-rank test. Univariate and multivariable Cox-regression analyses were used to determine independent prognostic factors for MSS and DFP.

Results

A total of 252 patients were selected for this study. Of these patients 173 were FDG-PET and CT negative (AJCC stage III) and 79 patients were positive (AJCC stage IV). Their median age was 57 years (range 19-93) and 99 were female (39%). Characteristics of the FDG-PET and CT negative and positive patients and their melanoma are shown in Table 1. No significant differences between both groups were found except for Breslow thickness ($P=0.02$) and TLND ($P<0.001$).

The median follow-up period was 33 months (range 6-112) for FDG-PET and CT negative and 12 months (range 1-99) for positive patients.

Survival complete cohort of patients

For the complete cohort 2- and 5-yrs MSS were 54.9% (95%-CI: 48.9-60.9%) and 38.2% (95%-CI: 32.2-44.2%), respectively (Figure 1). In the multivariable analysis positive FDG-PET and CT, the presence of extranodal growth and male gender were independently associated with worse MSS (Table 2). Patients presenting with LNM in the head and neck area tended to have a better MSS compared to those with metastases located in the axilla and groin, although the result compared to the groin was not significant (Table 2).

Table 1. Patient and melanoma characteristics of FDG-PET and CT negative and positive patients

Characteristics		FDG-PET and CT-	FDG-PET and CT+	P-value
Sex	Male	106 (61)	47 (59)	0.79
	Female	67 (39)	32 (41)	
Age*	Median (range)	57 (26-93)	58 (19-83)	0.81
	< 50	51 (29)	27 (34)	0.53
	50-64	72 (42)	27 (34)	
	>65	50 (29)	25 (32)	
Location melanoma	Arm	16 (9)	10 (13)	0.33
	Leg	67 (39)	21 (27)	
	Trunk	60 (35)	34 (43)	
	Head/neck	21 (12)	8 (10)	
	Unknown	9 (5)	6 (7)	
Breslow thickness	Median (range)	2.1 (0.45-15)	3.0 (0.67-13)	**0.02**
	T1 (≤1.00mm)	24 (14)	6 (7)	0.24
	T2 (1.01-2.00mm)	57 (33)	20 (25)	
	T3 (2.01-4.00mm)	48 (28)	29 (37)	
	T4 (>4.00mm)	30 (17)	14 (18)	
	Unknown	14 (8)	10 (13)	
Clark	Clark I/II	21 (11)	7 (9)	0.13
	Clark III	39 (23)	17 (22)	
	Clark IV	84 (49)	32 (40)	
	Clark V	14 (8)	12 (15)	
	Unknown	15 (9)	11 (14)	
Ulceration	Absent	130 (75)	50 (64)	0.17
	Present	33 (19)	20 (25)	
	Unknown	10 (6)	9 (11)	
Location node metastasis	Head/neck	32 (19)	11 (14)	0.06
	Axilla	56 (32)	38 (48)	
	Groin	85 (49)	30 (38)	
Tumor-containing LNs	Median (range)	2 (1-24)	2 (1-11)	0.92
Nstage	N1	72 (42)	20 (25)	0.76
	N2	40 (23)	14 (18)	
	N3	47 (27)	12 (15)	
	Unknown	14 (8)	33 (42)	
Lymph nodes removed	Median (range)	14 (1-48)	12 (1-29)	0.06
	≤10	43 (25)	17 (21)	0.12

	>10	114 (66)	26 (33)	
	Unknown	16 (9)	36 (46)	
Ratio of involved/total nodes (in %)	Median (range)	14 (2.8-100)	24 (3.9-100)	0.07
Tumorsize	Median (range)	2.8 (0-8)	3 (0.5-13)	0.17
	≤3	81 (47)	20 (25)	0.66
	>3	62 (36)	18 (23)	
	Unknown	30 (17)	41 (52)	
Extranodal growth	No	122 (70)	13 (16)	0.73
	Yes	41 (24)	34 (43)	
	Unknown	10 (6)	32 (41)	
Time of LNM	Synchronous presentation	75	26	0.12
	After presentation of PM	89	47	
	Unknown	9	6	
TLND	No	0	34	**<0.001**
	Yes	173	45	
Location metastases	Skin		11	
	Liver		19	
	Lung		33	
	Abdomen		15	
	Bone		26	
	Other		16	
Number of metastases	Single metastasis		48	
	Multiple metastases		31	
No. of patients		173 (69)	79 (31)	

*Age at time of presentation with palpable lymph node metastasis. LN: Lymph Node. LNM: Lymph Node Metasasis. PM: Primary Melanoma. TLND: Therapeutic Lymph Node Dissection.

Survival rates of FDG-PET and CT negative and positive melanoma patients

The 2-year MSS was 67.3% (95%-CI: 59.3-75.3%) for FDG-PET and CT negative patients and 25.8% (95%-CI: 15.8-35.8%) for FDG-PET and CT positive patients (P<0.001). The 5-year MSS was 47.6% (95%-CI: 39.6-55.6%) for the FDG-PET and CT negative patients and 16.9% (95%-CI: 6.9-26.9%) for FDG-PET and CT positive patients (P<0.001) (Figure 1).

In multivariable analysis for the FDG-PET and CT negative patients, males had worse MSS compared to females. Greater size of LNM and presence of extranodal growth were also independent factors for

worse MSS (Table 3). Patients with LNM in the head or neck had significantly better MSS than those with LNM in the axilla but there was no significant difference between LNM in head or neck and groin (Table 3).

In multivariable analysis for FDG-PET and CT positive patients only presence of extranodal growth was an independent factor for worse MSS (HR: 2.61, 95%-CI: 1.27-5.35, P=0.009). In 45 of the 79 FDG-PET and CT positive patients a lymph node dissection was performed, which had no significant impact on MSS of these patients (HR: 0.66, 95%-CI: 0.39-1.13, P=0.12). The number of distant metastases, as defined with FDG-PET and CT, was a significant prognostic factor in the univariate analysis (HR: 1.73, 95%-CI: 1.01-2.95, P=0.044), but in the multivariable analysis this was a trend (HR: 1.24, 95%-CI: 0.62-2.49, P=0.055). Location of distant metastases was not a significant prognostic factor in the univariate analysis (HR: 1.21, 95%-CI: 0.98-1.28, P=0.093).

Table 2. Multivariable Cox regression analysis of several melanoma factors on melanoma specific survival in all patients

Characteristic		**MSS**		
		HR	**95%-CI**	**P-value**
Gender	Male	1		
	Female	0.58	0.37-0.90	**0.016**
Conclusion FDG-PET and CT	Negative	1		
	Positive	2.54	1.55-4.17	<0.001
Ulceration	Absent	1		
	Present	1.47	0.93-2.32	0.10
Location LNs	Head/neck	1		
	Axilla	2.03	1.03-4.00	**0.043**
	Groin	1.75	0.91-3.38	0.10
Tumor-containing LNs	Continuous	1.03	0.98-1.08	0.23
Tumorsize	Continuous	1.06	0.97-1.16	0.21
Extranodal growth	Absent	1		
	Present	2.38	1.56-3.63	**<0.001**
Time of LNM	Synchronous presentation	1		
	Metachronous presentation	0.67	0.43-1.04	0.07

LN: Lymph Node. LNM: Lymph Node Metasasis. PM: Primary Melanoma. HR: Hazard Ratio. CI: Confidence Interval

Table 3. Multivariable cox regression analysis of several melanoma factors on melanoma specific survival in FDG-PET and CT negative patients

Characteristic		MSS		
		HR	95%-CI	P-value
Gender	Male	1		
	Female	0.53	0.31-0.89	**0.017**
Ulceration	Absent	1		
	Present	1.68	0.99-2.86	0.056
Location of LNM	Head/neck	1		
	Axilla	2.22	0.22-0.91	**0.027**
	Groin	1.68	0.43-1.33	0.188
Tumor-containing LNs	Continuous	1.04	0.99-1.09	0.117
Tumorsize	Continuous	1.21	1.07-1.38	**0.003**
Extranodal growth	Absent	1		
	Present	1.76	1.08-2.85	**0.024**
Time of LNM	Synchronous presentation	1		
	Metachronous presentation	0.61	0.37-1.01	0.057

LN: Lymph Node. LNM: Lymph Node Metasasis. PM: Primary Melanoma. HR: Hazard Ratio. CI: Confidence Interval

Recurrence rates in FDG-PET and CT negative patients

Disease free period for the 173 FDG-PET and CT negative patients was 55.8% (95%-CI: 47.8-63.8%) after 2-years and 46.0% (95%-CI: 38.0-54.0%) after 5 years. Ninety patients developed a recurrence (52%). Of these patients 12 developed only an in-field regional recurrence (13%), 60 patients developed only distant metastasis (67%), and 18 patients developed both regional and distant recurrence (20%). Of the 78 patients with distant recurrences, 16 patients (21%) developed metastases in multiple locations and 62 patients had a solitary metastasis (79%). The locations of distant recurrences were 22 skin or subcutis; 7 bone; 12 liver; 6 abdominal; 21 lung; 18 brain; and 6 other metastases.

In multivariable analysis presence of ulceration, greater size of LNM and metachronous presentation of LNM with primary melanoma were independent factors for worse DFP in FDG-PET and CT negative patients (Table 4).

The presence of extranodal growth was the only factor that significantly predicted regional recurrence after TLND (HR: 3.14, 95%-CI: 1.34-7.34, P=0.008). Only presence of ulceration was an independent predictive factor for distant metastases in FDG-PET and CT negative patients after TLND (HR: 2.23, 95%-CI: 1.23-3.83, P=0.004).

Table 4. Multivariable Cox regression analysis of several melanoma factors on disease free period in FDG-PET and CT negative patients

Characteristic		DFP		
		HR	95%-CI	P-value
Sex	Male	1		
	Female	0.69	0.40-1.18	0.178
Ulceration	Absent	1		
	Present	2.11	1.22-3.64	**0.008**
N-classification	N1b	1		
	N2b	0.78	0.41-1.50	0.46
	N3	1.54	0.88-2.72	0.13
Tumorsize	Continuous	1.26	1.10-1.44	**0.001**
Extranodal growth	Absent	1		
	Present	1.66	1.00-2.80	0.057
Time of LNM	Synchronous presentation	1		
	Metachronous presentation	0.53	0.32-0.88	**0.014**

LN: Lymph Node LNM: Lymph Node Metasasis. PM: Primary Melanoma. HR: Hazard Ratio. CI: Confidence Interval.

Discussion

Survival

In the present study the 5-year survival for all patients that underwent TLND for palpable LNM (38.2%) was similar to other studies (26-43%).[4,6-9] The 5-year survival in FDG-PET and CT negative patients (47.6%) is better compared to results of these previous studies (26-43%).[4,6-9] The 5-year MSS is worse in FDG-PET and CT positive patients (16.9%), but these patients had better survival compared to other recent studies on stage IV melanoma patients (range 4.9-11%).[11-14] This is due to the fact that distant metastases were found earlier in the present study, as FDG-PET and CT were performed directly after diagnosis of macrometastases in the lymph nodes. Hence, patients underwent FDG-PET and CT because of palpable lymph nodes and not because they presented with symptoms of distant metastases (stage migration). Therefore, adding FDG-PET and CT to the diagnostic work-up for melanoma patients who present with palpable LNM improves taxonomy of these patients so these patients can be subdivided into new staging groups.

The subdividing of clinically stage III melanoma patients into new groups using FDG-PET/CT is important. Firstly, to identify those patients who may benefit from a surgical procedure, while avoiding these potentially harmful surgical procedures for patients with multiple distant disease. With the use of a FDG-PET/CT before TLND, a better selection of patients can be made to distinguish between curative

or palliative lymph node dissection with or without adjuvant radiation. Moreover, patients where distant metastases are found on the FDG-PET/CT might benefit from other treatment options, including surgery, drug-targeting therapy, immunotherapy and/or chemotherapy. Especially in the era where both surgical management and management with systemic therapies of stage IV melanoma patients showed promising results and trials for adjuvant systemic therapies of stage III patients are initiated, FDG-PET/CT would be a valuable addition to the diagnostic work-up of clinically stage III patients, so every patient will receive a patient-tailored treatment.[15,18] In addition, incidental findings are found on FDG-PET and CT in 4.3% and can also alter management of clinically stage III patients.[19]

FDG-PET and CT staged IV melanoma patients should also be evaluated with MRI of the brain because the likelihood of detecting additional asymptomatic lesions is higher compared to stage III melanoma patients (12% versus 1.6%) and management of stage IV patients can change due to the detection of brain metastases in these patients.[20-22] Finally, improved taxonomy of clinically stage III patients using FDG-PET/CT is important to improve efficacy of clinical trials due to more homogeneous patient groups and to provide patients with accurate information about their prognosis.

Patients underwent both FDG-PET and CT as the previous study by Bastiaannet et al[17] was designed to compare both these imaging modalities. However, nowadays most centers have an integrated FDG-PET/CT, this combined imaging modality is preferred compared to FDG-PET and CT separately. Although FDG-PET and CT are expensive imaging modalities, adding FDG-PET and/or CT to the diagnostic work-up of clinically stage III melanoma patients does not increase costs substantially.[23] The cost-effectiveness of combined FDG-PET/CT is yet to be studied. However, results of such a study are expected to be similar compared to the study of Bastiaannet et al.[23]

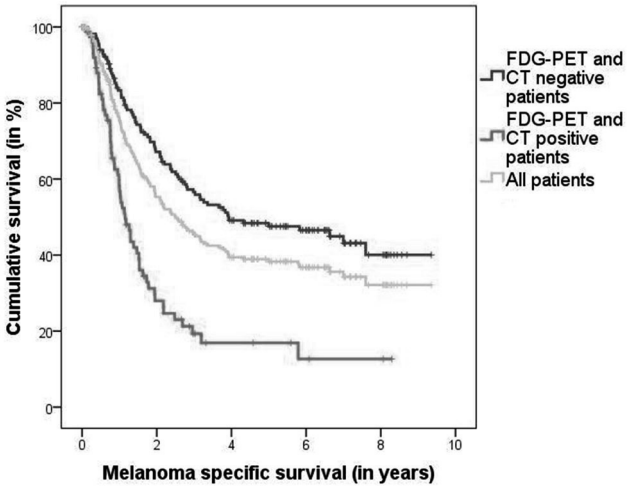

Figure 1. Melanoma specific survival for all patients, FDG-PET negative and positive patients.

Factors with impact on survival

In all patients, gender; FDG-PET and CT; location and extranodal growth of lymph nodes were independent prognostic factors for MSS. A positive FDG-PET and CT was the prognostic factor with the highest Hazard Ratio (HR: 2.54, 95%-CI: 1.55-4.17, P<0.001). This result shows that FDG-PET and CT is the most important prognostic factor in clinical stage III melanoma patients and therefore these scans have a prognostic value in addition to the other prognostic factors.

In FDG-PET and CT negative patients gender was a significant independent prognostic factor for MSS, localization, size, and extranodal growth of LNM were also independent prognostic factors for MSS. Several other studies have shown worse prognosis for males with melanoma.[24,25] Other studies have shown that increasing size of lymph nodes and presence of extranodal growth are associated with a worse prognosis.[9,26-38] The amount of positive nodes was not an independent prognostic factor for both MSS and DFP. In other recent studies the number of positive lymph nodes was also not seen to be a prognostic factor for overall or disease free survival.[32,39] Head/neck LNM had significantly better MSS than LNM in the axilla, MSS was also better in head or neck compared to groin LNM, although this was a trend. The results in recent literature about the impact on lymph node location on overall survival are contradictive as two studies showed worse prognosis for head/neck lymph nodes[32,40] and two other studies showed better prognosis for head/neck LNM.[9,41] However, the first of the latter two studies is from one of the centers that participated in the present study (University Medical Centre Groningen) with a cohort of patients that was partly similar.[9] Nevertheless, the present study confirms this result in multiple centers.

Primary melanoma characteristics were not to be associated with overall survival in clinical stage III or stage IV patients, which is similar to the results of other studies.[4,8,9] In the present study the only significant independent prognostic factor for survival of worse MSS in stage IV melanoma patients was the presence of extranodal growth. Several studies showed the location and number of metastases to be factors that significantly influence survival of stage IV melanoma patients.[4,15,16] However, in the present study number of metastases was a trend in the multivariable analysis and location of metastases was a trend in the univariate analysis. TLND did not have a significant survival benefit for stage IV melanoma patients. Therefore with the use of the FDG-PET or CT in clinical stage III melanoma patients before performing TLND, patients who are staged with distant metastases could be spared from TLND and its possible complications.

Recurrences

The 5-year DFP in FDG-PET and CT negative patients was 46% (95%-CI:41.4-50.2%), this is significantly higher than DFP of the complete cohort of patients. DFP is higher than what has been reported in patients with palpable lymph nodes (19-27%).[7,9,10] In FDG-PET and CT negative patients, factors that had impact on worse DFP were presence of ulceration, greater size of LNM, and metachronous presentation of LNM and primary melanoma. Ulceration was also the only independent predictive

factor for distant metastases recurrences. Two studies have also shown that ulceration was a predictive factor for distant metastases recurrence.[12,42] Ulceration was not a predictive factor for regional recurrence, this is also found in other studies,[43,44] although this result is in contrast with the study of Soong et al.[45] Greater size of LNM has already been shown to predict regional recurrence in stage III melanoma patients.[9,32,46] Synchronous presentation of melanoma and lymph node metastasis showed better survival in a study of White et al.[47] However, in that study regional metastases from unknown primary sites (with their observed survival benefit) were considered to be synchronous. Moreover, a presentation on ASCO in 2008 showed better survival for metachronous presentation.[48] Which would be more logical as presentation of LNM synchronous with a primary melanoma seems to be a much more aggressive tumor. Furthermore, Murali et al recently showed that greater time to recurrence is a predictive factor for better post recurrence survival.[44]

In the total follow-up period 90 patients who were initially FDG-PET and CT negative (52%) developed a recurrence, of which 13% was loco-regional, 67% distant metastasis recurrence, and 20% had both loco-regional and distant metastasis recurrence. Also, in other studies the majority of the recurrences in stage III patients were distant recurrences after TLND.[9,10] The majority of distant recurrences were skin or subcutis and lung metastases. Romano et al. had a similar result in stage IIIC melanoma patients.[10]

Conclusion

In conclusion, melanoma patients with palpable LNM can be subdivided into more accurate prognostic groups using whole body FDG-PET and CT added to the diagnostic work-up. Hence, this study confirms that FDG-PET and CT, preferably combined as an integrated imaging modality, are indicated in the staging of melanoma patients with palpable LNM. The present study also shows an improvement in survival for stage III and IV melanoma patients as compared to historical cohorts; this stage migration has to be taken into account in further studies on survival time trends. The main importance of the improved taxonomy of clinically staged III patients is that every patient will get a so called patient-tailored treatment according to their stage of disease.

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Chapter 10

Summary and conclusions

The incidence of melanoma is rising worldwide.[1] Due to the unpredictability and erratic dissemination of melanomas, its management is challenging. It is currently almost impossible to cure distant disseminated melanoma patients, although new therapies , including immunotherapy and drug targeted therapies, offer hope for the future.[2-4] Consequently, accurate staging is extremely important for clinical decision making to identify those patients that need surgical, systemic and/or radiation treatment while avoiding potentially harmful therapies that does not improve the patients survival or quality of life. The present chapter discusses the different leads generated by this thesis to improve accuracy on staging melanoma patients and therefore improve treatment outcome.

Pathology of melanoma

Early detection, accurate diagnosis and appropriate treatment of primary melanoma when it is at an early clinical stage are crucial for melanoma disease control. An accurate melanoma histopathology report that documents important pathologic features is essential for accurate staging, predicting prognosis and guiding the next stages of the patient's management. *Chapter 2* revealed that reproducibility of important, T-classification, pathologic features is excellent and is improved in the last ten years. Furthermore, the completeness of pathology reports for important pathologic features has also improved in recent years, especially for pathologists outside a specialist melanoma treatment center. This is probably due to the more frequent utilization of structured pathology reports. *Chapter 2 and 12* show an example of a structured pathology report.

Although reproducibility of pathologic features has improved, still many pathologists disagree on the histopathologic diagnosis/staging of melanoma. *Chapter 3* showed that diagnosis, T-stage, and therefore management recommendations for surgical excision margins/sentinel lymph node biopsy, often change following pathology review by a pathologist at a specialist melanoma treatment center. In addition, after review completeness of the pathology report increases significantly. Therefore pathology review should be considered for all patients attending specialist melanoma treatment centers. *Chapter 2* showed that documentation for most important pathologic features is excellent. However, the documentation of microsatellites is poor, especially in pathology reports from outside a specialist melanoma treatment center. This is disappointing since *Chapter 4* showed that the presence of microsatellites is an important prognostic factor. The 5-year survival declines from 67.4% to 42.8% when microsatellites are present around the primary tumor. Furthermore, microsatellites are associated with locoregional recurrences and sentinel lymph node positivity.

Sentinel lymph node biopsy

The status of the sentinel lymph node is the most important predictor of prognosis and tumor recurrence for early melanoma and a sentinel lymph node biopsy is a widely accepted diagnostic procedure. This procedure is currently most often performed using a combination of blue dye and radiotracer in order to guide the surgeon to the sentinel lymph nodes. However, this procedure can also

be performed using solely blue dye, solely radiocolloid, indocyanine green, and indocyanine green and radiocolloid combined. *Chapter 5* showed that in breast carcinoma/melanoma patients sentinel lymph node identification rates are high for all these techniques. Furthermore, the false negative rates for all these techniques are low. However, the identification rate for sentinel lymph node biopsy using solely radiocolloid or radiocolloid combined with blue dye was found to be significantly higher compared to using solely blue dye. Furthermore, in the present era, the addition of blue dye to radiotracers does not increase the sentinel lymph node identification rate. Taken together with the side effects of blue dye, we advise surgeons, experienced with the radiocolloid sentinel lymph node biopsy technique, working in hospitals in the Netherlands to perform sentinel lymph node biopsy using solely radiocolloid. In the Netherlands radiotracers are, although expensive, available in most hospitals. However, in developing countries availability of radiotracers are limited. Therefore lymphatic mapping using solely blue dye should be encouraged in hospitals where radiotracers are not available, since the identification rate for using solely blue dye is acceptable.
Finally, performing a sentinel node biopsy using indocyanine green as a fluorescent dye seems a promising technique for the near future.

Treatment of melanoma patients with lymph node metastases

Melanoma patients with regional metastases detected by sentinel lymph node biopsy are in general treated with a Completion Lymph Node Dissection (CLND) or monitored with ultrasound in the Multicenter Selective Lymphadenectomy Trial II (MSLT-II).[5] Patients with clinical disease are first staged using FDG-PET/CT (Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography), when no metastatic disease is encountered these patients are treated by a so called Therapeutic Lymph Node Dissection (TLND) with or without adjuvant systemic and/or radiation therapy.[6,7] Melanoma patients with lymph node metastases in the groin mostly have superficial (inguinal) metastases, however, *Chapter 6* showed that deep (iliac or obturator) groin metastases are also fairly common, even in sentinel lymph node positive patients. The presence of metastases in the deep lymph nodes is an important prognostic marker, as they tremendously affect prognosis, particularly in sentinel lymph node positive patients. The poor prognosis of deep lymph node metastases could be an indication for the use of adjuvant systemic and/ or radiation therapy. As explained in *Chapter 7*, new systemic drugs, including BRAF and MEK inhibitors might be beneficial as adjuvant systemic treatment in melanoma patients with only lymph node metastases. The combination of BRAF and MEK inhibitors in stage IV melanoma patients has already shown promising results, although the durability of the drug effect was limited.[3] In stage III patients this combination of drugs might induce longer efficacy as tumor load is low compared to stage IV patients, especially when lymph node dissection has already been performed. The value of the combination of BRAF and MEK inhibitors in stage III melanoma patients is currently tested in a placebo-controlled trial, the COMBI-AD trial.[8]

Imaging in melanoma patients

Accurate staging is extremely important for clinical decision making to identify those patients who may benefit from surgery, while avoiding unnecessary, potentially harmful surgery that does not improve survival. Furthermore, accurate staging is important to properly select patients for trials and patient counseling on prognosis. Imaging with computed tomography (CT), magnetic resonance imaging (MRI) and especially molecular imaging play an important role in staging of melanoma patients. *Chapter 8* discussed the value of these imaging modalities, with particular emphasis on nuclear imaging.

Sentinel lymph nodes are currently detected with the use of sentinel lymph node biopsy; this procedure is assisted by preoperative lymphoscintigraphy with ^{99m}Technetium as a radiotracer for localizing sentinel lymph nodes. For the detection of distant metastases a FDG-PET /CT is mostly used. This radiopharmaceutical is very effective for localizing melanoma cells, as these are typically FDG-avid. However, uptake of FDG is also seen in inflammation, infection, and is also taken up by muscles and the central nervous system. Furthermore, FDG-PET sensitivity is lower in detecting melanoma foci in lung, liver and brain. New radiopharmaceuticals specific for melanomas, including [18F]ICF01006, may offer better capacity for further PET diagnostics.

In stage I and II, and microscopic stage III patients FDG-PET/CT has no additional value. However, *Chapter 9* revealed that for patients with palpable, cytologically proven lymph node metastases with no suspicion for lung metastases on X-ray, FDG-PET/CT does have additional value in treatment planning. With FDG-PET/CT added to the diagnostic work-up, clinical stage III patients can be subdivided into more accurate prognostic groups. The main importance of the improved taxonomy of these patients is that every patient will get a so-called patient-tailored treatment according to their stage of disease. Also, for stage IV melanoma patients FDG-PET/CT may be of importance to localize the distant metastases if surgical treatment is considered. FDG-PET/CT staged IV melanoma patients should also be evaluated with MRI of the brain because the likelihood of detecting additional asymptomatic lesions is high (12%) and management of stage IV patients can change due to the detection of brain metastases in these patients.

In conclusion, melanoma remains a tumor with ‘unpredictable behavior’, which makes its staging and management challenging. However, this thesis generated different leads that contribute to improving the accuracy of staging in patients with primary and metastatic melanoma. Through the improvement of staging accuracy and therefore the improved understanding of the biological behavior, melanomas are becoming more ‘predictable’. Due to the accurate staging patients will be informed about their prognosis more accurately and medical multidisciplinary teams will be able to treat melanoma patients with more suitable, patient-tailored, therapies.

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Chapter 11

Nederlandse samenvatting en conclusies

De incidentie van het melanoom stijgt wereldwijd.[1] Door de onvoorspelbare en onregelmatige disseminatie van het melanoom is de behandeling uitdagend. Hedendaags is het nog steeds vrijwel onmogelijk om een patiënt met een op afstand gemetastaseerd melanoom te genezen. Nieuwe therapieën, zoals immunotherapie en doelgerichte therapieën geven echter hoop voor de toekomst.[2-4] Om therapeutische beslissingen te maken is een accurate stadiëring van het melanoom nodig. Hierdoor kan onderscheid worden gemaakt tussen patiënten die baat hebben bij een chirurgische, of adjuvante systemische en/of radiotherapeutische behandeling en patiënten die geen baat hebben bij deze behandelingen. In dit hoofdstuk worden verschillende aanknopingspunten die dit proefschrift voortbrengt op een rij gezet en hun bijdrage aan het verbeteren van de diagnostiek en behandeling van melanoom patiënten.

Pathologie van het melanoom

Vroege detectie, accurate diagnose en een passende therapie van het primaire melanoom in een vroeg stadium is van cruciaal belang voor het controleren van deze ziekte. Een nauwkeurig en compleet pathologieverslag van het melanoom is essentieel voor een nauwkeurige stadiëring en het voorspellen van de prognose. Het vormt een leidraad voor de behandeling van de patiënt met een melanoom. *Hoofdstuk 2* toont dat de reproduceerbaarheid van belangrijke pathologische factoren in de T-classificatie uitstekend is en dat dit verbeterd is in het laatste decenium. Bovendien is in deze periode de compleetheid van pathologieverslagen voor belangrijke pathologische eigenschappen van het melanoom toegenomen, voornamelijk van pathologen die in een niet-specialistisch centrum werken. Dit komt hoogstwaarschijnlijk doordat er steeds meer gebruik gemaakt wordt van gestructureerde pathologieverslagen, waarvan in *Hoofdstuk 2 en 12* een voorbeeld wordt gegeven.

Ondanks de toegenomen reproduceerbaarheid van de diagnose en pathologische eigenschappen van het melanoom blijft de pathologie van het melanoom subjectief en zijn veel pathologen het hierover oneens met elkaar. *Hoofdstuk 3* laat zien dat in veel gevallen de diagnose, stadiëring, en daardoor de richtlijnen voor chirurgische behandeling of schildwachtklierprocedure, veranderen als een gespecialiseerde patholoog het pathologieverslag van een externe patholoog herziet. Bovendien verbetert de compleetheid van het pathologieverslag significant na het herzien van het pathologieverslag. Om deze reden dient het pathologieverslag altijd te worden herzien door een patholoog van een specialistisch centrum indien de patiënt hiernaar verwezen wordt.

Hoofdstuk 2 liet weliswaar zien dat de documentatie van de meeste belangrijke pathologische factoren uitstekend is, maar de documentatie van microsatellitose blijft matig, helemaal in pathologieverslagen buiten een specialistisch centrum. Dit is teleurstellend aangezien *Hoofdstuk 4* laat zien dat de aanwezigheid van microsatellitose een belangrijke prognostische factor is. De 5-jaarsoverleving daalt namelijk van 67.4% naar 42.8% bij de aanwezigheid van microsatellitose rondom de primaire tumor. Bovendien is microsatellitose geassocieerd met een positieve schildwachtklier en locoregionale recidieven.

Schildwachtklierbiopsie

De schildwachtklierbiopsie is een wereldwijd geaccepteerde diagnostische procedure en de status van de schildwachtklier is de belangrijkste voorspeller voor de prognose en tumor recidief van het melanoom. Deze procedure wordt tegenwoordig voornamelijk uitgevoerd met een combinatie van blauwe kleurstof en een radioactieve stof (radiocolloid) om de chirurg te leiden naar de schildwachtklieren. *Hoofdstuk 5* laat zien dat deze procedure ook nauwkeurig is uit te voeren door alleen een radiocolloid te gebruiken, vooral als de chirurg ervaring heeft met de procedure. Mede door potentiële allergische reacties tegen de blauwe kleurstof, wordt in *Hoofdstuk 5* geadviseerd om de schildwachtklierprocedure met alleen het radiocolloid uit te voeren in landen (zoals Nederland) waar dit beschikbaar is. Voor landen waar radiocolloid niet beschikbaar is, zou de procedure ook met alleen de blauwe kleurstof of indocyanine groen uitgevoerd kunnen worden, vooral indocyanine groen liet in *Hoofdstuk 5* veelbelovende resultaten zien voor de toekomst.

Behandeling van melanoom patiënten met lymfeklier metastasen

Melanoom patiënten met regionale metastasen die ontdekt zijn met behulp van een schildwachtklierbiopsie worden over het algemeen behandeld met een 'Completion Lymph Node Dissection' (CLND) of worden gemonitord met behulp van een echografie in de 'Multicenter Selective Lymphadenectomy Trial II' (MSLT-II).[5] Patiënten met klinisch palpabele lymfeklier metastasen worden eerst gestadiëerd met een FDG-PET/CT (Fluorodeoxyglucose Positron Emissie Tomografie/Computed Tomografie). Als hier geen afstandsmetastasen op worden aangetoond, dan wordt de patiënt behandeld met een 'Theurapeutic Lymfe Node Dissection' (TLND) met of zonder adjuvante systemische therapie en/of radiotherapie.[6,7] Melanoom patiënten met lymfeklier metastasen in de lies hebben meestal oppervlakkige (inguinale) metastasen. *Hoofdstuk 6* laat echter zien dat ook in diepe (iliacaal of obturator) gelegen lymfeklier vaak metastasen aanwezig zijn, zelfs in patiënten waarbij de lymfekliermetastasen nog niet palpabel waren. De aanwezigheid van diep gelegen liesklier metastasen is een belangrijke prognostische marker, omdat dit de overleving sterk beïnvloedt, vooral in patiënten waarbij de lymfekliermetastasen nog niet palpabel waren. De slechte prognose van diep gelegen liesklier metastasen zou een indicatie kunnen zijn voor het geven van adjuvante systemische therapie en/of radiotherapie, bijvoorbeeld een therapie met BRAF en MEK remmers. De combinatie van BRAF en MEK remmers heeft al veel belovende resultaten laten zien in stadium IV melanoom patiënten, hoewel de duur van het effect van de medicijnen beperkt was.[3] In stadium III patiënten zouden deze medicijnen juist een langdurige werking kunnen hebben, omdat in deze patiënten de 'tumor load' relatief lager is, helemaal als de lymfeklierdissectie al is uitgevoerd. De waarde van de gecombineerde behandeling van BRAF en MEK remmers in stadium III melanoom patiënten wordt momenteel onderzocht in een placebo gecontroleerde studie, de COMBI-AD trial.[8]

Beeldvorming in melanoom patiënten

Beeldvorming met CT, magnetische resonantie imaging (MRI) en vooral moleculaire beeldvorming spelen een belangrijke rol in de stadiëring van patiënten met een gemetastaseerd melanoom. *Hoofdstuk 8* bespreekt de waarde van deze beeldvormende technieken, waarin vooral de rol van moleculaire beeldvorming bij het melanoom wordt besproken. Schildwachtklieren worden tegenwoordig ontdekt met een schildwachtklierbiopsie. Deze procedure wordt uitgevoerd met behulp van preoperatieve lymfoscintigrafie met ^{99m}Technetium als leidraad voor het lokaliseren van de lymfeklieren.

Voor de detectie van afstandsmetastasen wordt FDG-PET/CT vaak gebruikt. FDG is zeer effectief in het lokaliseren van melanoomcellen, aangezien deze erg FDG-gevoelig zijn. FDG wordt echter ook opgenomen bij ontsteking, infectie en door spiercellen en cellen van het centrale zenuwstelsel. Derhalve is de sensitiviteit van FDG-PET lager in long, lever en brein in het detecteren van melanoom metastasen. Nieuwe melanoom specifieke 'radiotracers' , zoals [18F]ICF01006, lijken de sensitiviteit van de PET/CT voor het opsporen van melanoom metastasen te verhogen.

In stadium I en II en microscopisch stadium III melanoom patiënten heeft FDG-PET/CT geen toegevoegde waarde. In *hoofdstuk 9* bleek echter dat voor patiënten met palpabele, cytologisch bewezen lymfekliermetastasen zonder aanwijzing voor long metastasen op de röntgenfoto van de thorax, FDG-PET/CT wel toegevoegde waarde heeft voor de vervolg behandeling. Met FDG-PET/CT toegevoegd aan de diagnostische work-up, kunnen patiënten met palpabele lymfekliermetastasen onderverdeeld worden in meer accurate prognostische groepen. Het voornaamste belang van de verbeterde taxonomie in stadium III patiënten is dat iedere patiënt een op maat gemaakte behandeling krijgt.

Ook voor stadium IV melanoom patiënten kan FDG-PET/CT van belang zijn om metastasen op afstand te lokaliseren indien een chirurgische behandeling wordt overwogen. Stadium IV patiënten die met FDG-PET/CT gestadieerd zijn dienen ook een MRI van het brein te ondergaan. De kans op het detecteren van bijkomende asymptomatische laesies is hoog (12%) en behandeling van deze groep patiënten veranderd zodra hersenmetastasen gedetecteerd worden.

Concluderend blijft het melanoom een onvoorspelbare tumor met een onregelmatig metastaseringspatroon, waardoor de behandeling van het melanoom uitdagend blijft. Dit proefschrift beschrijft verschillende aanknopingspunten die kunnen bijdragen aan een accuratere stadiëring van patiënten met een primair of gemetastaseerd melanoom. Hierdoor wordt het steeds duidelijker hoe het melanoom zich biologisch gedraagt en krijgen patiënten een nauwkeurige prognose. Bovendien kunnen medische multidisciplinaire teams melanoom patiënten beter behandelen met een geïndividualiseerde behandeling.

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Chapter 12

Future perspectives

Rising incidence and prevention of melanoma

Melanoma incidence rates have almost doubled in the last decade with 2791 cases of melanoma in 2002 and 5084 in 2011 in the Netherlands.[1] Furthermore, it is expected that this incidence rate will further rise to approximately 5400 cases in 2020.[2]

Given the increasing incidence of melanoma, it is important to understand its economic burden. A recent German study showed that the annual per-patient medical costs increases for patients with more advanced melanoma (Table 1).[3] Due to the increasing costs of medical care for melanoma patients and the development of new promising, yet expensive, systemic medical agents costs for melanoma are expected to rise exponentially.

Table 1. The annual per-patient medical costs for each stage of melanoma shown by Seidler et al.[3]

AJCC stage of melanoma	Annual costs in $
In situ melanoma	8930
Stage I	14499
Stage II	26667
Stage III	31778
Stage IV	39631

The increasing costs are best opposed by primary prevention of melanoma. Primary prevention should be aimed at protection against ultraviolet (UV) radiation, from the sun or tanning beds, as this is the primary cause of most melanomas.[4-7] UV radiation exposure in childhood appears to be associated with a higher relative risk of melanoma than in later life, especially UV radiation from tanning beds.[4,5,7] Therefore both European and US law state that the use of tanning beds is prohibited for people younger than 18 years old. In 2010, a 10% tax was introduced on the use of tanning beds in the US, after the introduction of this tax, tanning bed use declined by 24%.[8] European countries are currently debating whether a tax on the use of tanning beds should be introduced.

Patient education

Early detection of melanoma is one of the most important factors for successful management, allowing treatment to be undertaken when cure is still achievable. Patient education is very important in the early detection of melanoma, since the majority of all melanoma recurrences are detected by the patient themselves.[9] Websites and social media are tools to educate patients and the University Medical Centre Groningen recently developed a melanoma website[10] with a comprehensive overview of all aspects of cutaneous melanoma. Furthermore, instructional videos, made in collaboration with the Dutch Cancer Society, are available on the website in order to assist patients in self -examination of the skin[11] and the lymph nodes.[12] Websites and social media are currently very important for patient education and are expected to play an even greater role in the future of medicine.[13]

Pathology of primary cutaneous melanoma

Pathologic parameters of the primary tumor are the strongest predictors of outcome in patients with clinically localized primary cutaneous melanoma.[14] Currently Breslow thickness, presence of ulceration and presence of tumor mitotic rate are the cornerstones of pathologic staging of cutaneous melanoma.[15]

Recently a study of 4661 patients showed that the extent of ulceration (measured in % or in mm) provides more accurate information regarding the prognosis than the mere presence of ulceration.[16] The study showed that the 5-year melanoma specific survival for minimally/moderately ulcerated melanomas (≤70% or ≤5 mm) was 80.4% and 82.7%, respectively, and for extensively ulcerated melanomas (>70% or >5 mm) 5-year survival was 66.4% and 59.3%.[16] Therefore the extent of ulceration might be included in future staging guidelines of primary cutaneous melanoma.

Another pathologic feature that was recently shown to be an independent prognostic indicator for melanoma is tumor infiltrating lymphocytes (TIL) (graded from 0 to 3, based on increasing extent and density of the TIL infiltrate).[17] This pathologic feature reflects a host immunologic response that may result in elimination of part or all of the melanoma (regression) and therefore the presence of TIL is associated with a favorable prognosis. In addition, a recent study also showed that the grade of TIL was associated with SLN positivity (grade 0=27.8%, 1=20.1%, 2=18.3%, 3=5.6%).[17] It is expected that this pathologic feature will play an important role in the staging of primary cutaneous melanoma.

An accurate and complete pathology report that documents all pathologic features is essential for guiding the melanoma patient's initial treatment (Table 2). Although the importance of melanoma diagnostic and staging criteria has been established, they remain subjective with significant interobserver variability between pathologists.[18-21] Also in the Netherlands a recent study showed the need for improvement in structured pathology reporting of melanoma.[22] Therefore efforts have been made to improve quality and completeness of melanoma pathology reports. In 2010 the Royal College of Pathologists of Australasia published a structured melanoma pathology reporting protocol in Australia, which is likely to improve quality of melanoma pathology reporting.[23] Furthermore, the efforts of the international pathology community to develop agreed uniform melanoma pathology reporting guidelines for implementation in each of their respective jurisdictions are likely to assist in this effort internationally.[24] Nevertheless, validation of success of these efforts in the future will be necessary to justify the considerable continued resource commitment that this entails.

Sentinel lymph node biopsy and lymph node dissection

Sentinel lymph node biopsy (SLNB) has already shown to be a highly accurate and minimally invasive staging technique in melanoma patients.[25] The question remains as to whether SLNB has a survival benefit for melanoma patients. The most recent interim analysis of the Multicenter Selective Lymphadenectomy Trial I (MSLT-I)showed that at 10-years, melanoma-specific survival for all randomized patients with trunk and extremity primaries was 78.1% for SLNB patients versus 71.0% for patients in

the observation group (p=0.046,Hazard Ratio (HR): 0.73).[26] However, the majority of these patients are SLN negative and could therefore never benefit from SLNB in terms of survival. Hence, the actual survival analysis should be performed in SLN positive patients and patients in the observation group who developed lymph node metastases. Comparing these two groups, the ten-year survival was significantly higher after immediate completion lymph node dissection (CLND) for SLN positive patients compared to patients of the observation group that underwent delayed CLND for clinical nodal recurrence (63.2% vs. 36.5%; HR: 0.49, P=0.001).[26] The final paper with the definitive data of the MSLT-I is accepted by the New England Journal of Medicine and will be published in 2014.

Melanoma patients with a positive sentinel lymph node are currently treated with a CLND or monitored with ultrasound in the MSLT-II trial.[27] The results of this trial and of the EORTC 1208 (Minitub) trial[28] will provide information about the possible survival benefit of CLND and about which patients are indicated for CLND and which for observation.

Imaging techniques in melanoma

PET/CT

Fluorodeoxyglucose-Positron Emission Tomography/Computed Tomography (^{18}F-FDG-PET/CT) is the most accurate imaging modality for the detection of distant metastases in melanoma.[29] However, due to the high physiological uptake of ^{18}F-FDG, the accuracy in detecting melanoma metastases in the liver and brain is limited.[30] Therefore new radiotracers are being developed to enhance signal-to-background ratios by improving targeting and/or capitalizing on lower background tracer uptake. Recent developments with melanotropin analogues and benzamide based tracers are described in the following section.

Melanotropin analogues (alpha-MSH)

Melanoma cells have high affinity to melanotropic hormone (alpha-melanocyte stimulating hormone). The hormone stimulating melanocytes act on the cell via the MelanoCortin type 1 Receptor (MC1R). MC1R is a G-protein coupled receptor which is over-expressed in many types of melanoma, making it an attractive target for receptor based melanoma imaging and therapy. Peptide based radioactive probes have been extensively studied for tumor receptor targeted imaging and therapy. When ^{18}F labeled α-MSH was used as a radiopharmaceutical, a-specific uptake prevailed in lung, liver and gallbladder.[31] However, recent research showed promising results of ^{18}F labeled α-MSH metallopeptides in vivo, with less lung and liver uptake compared to previous studies.[32] Nevertheless, further studies are required for further analysis of this peptide.

Benzamide based PET tracers

PET/CT is currently not recommended for staging in stage I, II, and microscopic stage III melanoma pa-

tients.[30,33,34] However, due to recent developments concerning benzamide derivates this might change in the near future. Although long uptake interval time (18-24h) and accumulation of the compound in the liver are drawbacks for benzamide derivates, one study showed sensitivity and specificity of 81% and 100%, respectively, for detection of distant metastases in melanoma patients.[35] Moreover, recent studies showed selective uptake with high tumor/non-tumor ratios of benzamide derivates and shorter uptake interval time (1-2h).[36,37] In an in vitro study the tumor-to-background ratio of benzamide ^{18}F-MEL050 was more than 9-fold higher compared to ^{18}F-FDG in a melanoma allograft model.[36,37] Recently the potential of benzamide ^{18}F-ICF01006 was evaluated as a PET tracer for early detection of melanoma lesions in mice where it showed to exhibit highly contrasted tumor imaging. In this study ^{18}F-ICF01006 showed subcutaneous metastases before they were even palpable.[38] Moreover, ^{18}F-ICF01006 was superior compared to ^{18}F-FDG with regard to contrast and specificity.[38] The high specificity of ^{18}F-ICF01006 for melanoma cells and its rapid elimination from non-target tissue allow an early detection of distant melanoma metastases in different sites of the body.[38] Although further studies analyzing benzamide derivates are warranted, the recent results have shown that they have high potential for early detection of melanoma metastases and this provides optimism for applying these as radiopharmaceuticals for melanoma in the future.

PET/MRI

Although accuracy of PET/CT in detecting distant melanoma metastases is currently high, it still has several limitations. For example, a major drawback is that CT provides only limited soft tissue contrast and exposes the patient to a significant radiation dose.[39] Secondly, PET/CT does not allow simultaneous data acquisition, since PET and CT scanners are hard-wired back to back and share a common patient bed.[39] This temporal mismatch causes image artifacts by patient movement between the two scans or by respiration motion.[39] To overcome these limitations, recent research concentrates on the combination of PET and MRI into one single machine. The goal of this development is to integrate the PET detectors into the MRI scanner which would allow simultaneous data acquisition, resulting in combined functional and morphological images with an excellent soft tissue contrast, very good spatial resolution of the anatomy and very accurate temporal and spatial image fusion.[39,40] First experiments with PET/MRI prototypes have shown very promising results, indicating its great potential for clinical and preclinical imaging.[39-41] Even PET/CT-MRI was tested as a trimodality for accurate detection of distant metastases.[42] The first PET/MRI has been placed in the Netherlands in October 2012, in the VU Medical Center. Data on the performance of PET/MRI in melanoma are awaited but not available at present, however, PET/MRI is expected to become an important tool for the detection of both lymph node and distant melanoma metastases.[43]

Optical imaging

Finally, there are current vibrant developments of optical imaging systems for intraoperative fluo-

rescence epi-illumination.[44] Optical imaging will not replace the previous described modalities but is an additional tool for improving staging and treatment of melanoma in the near future.[44,45] Optical imaging is a noninvasive, relatively low-cost technology that uses light to probe cellular and molecular function in the setting of cancer. This technology can enhance surgical vision of metastases in the operating room and during surgery. Advances in optical instrumentation allow the detection of endogenous tissue contrasts between normal and malignant tissues. Newer targeted optical imaging probes have been developed in preclinical animal models and can provide for better cancer cell detection.[45] Unfortunately, at present, no data is available for optical imaging in melanoma.

New systemic therapies

Recent studies have shown promising results using new systemic therapies in melanoma patients with distant metastases (stage IV). Systemic treatment with Ipilimumab, an anti-CTLA4 antibody, showed an increase of median overall survival in patients receiving ipilimumab from 6.4 to 10.0 months.[46] Furthermore, a study presented at the 2013 European Cancer Congres showed that a survival of more than 3-10 years could be achieved in 17-25% of patients with metastatic, or locally advanced, unresectable melanoma.[47] These survival results could even double or triple with anti-PD1/PDL1 monoclonal antibodies.[47]

Understanding the role of activation of the Mitogen-activated protein kinase (MAPK) pathway has led to the identification of several drug targets, including BRAF and MEK. Flaherty et al and Chapman et al showed promising results for BRAF inhibitors (Vemurafinib) in stage IV melanoma patients.[48,49] Moreover, Flaherty et al recently showed in a phase I and phase II trial that a combination of BRAF (Dabrafenib) and MEK inhibitors (Trametinib) compared to monotherapy with a BRAF inhibitor improved median progression-free survival from 5.8 to 9.4 months.[50] Currently a phase III trial has been initiated comparing a combination of BRAF and MEK inhibitors versus a BRAF alone in stage IV melanoma patients (Combi-v trial).[51] The future will see intensified efforts to map signal transduction pathways of melanoma cells and block these simultaneously with systemic medical agents in order to increase the durability of the therapeutic responses.

In addition, MAPK inhibitors are soon to be tested in stage III melanoma patients as adjuvant therapy. The upcoming COMBI-AD trial will study the effect of adjuvant systemic treatment with a BRAF inhibitor combined with a MEK inhibitor on relapse-free survival in a placebo controlled trial.[52] Patients eligible for this trial will be BRAF positive stage III melanoma patients who are surgically treated with curative intent. The main question of this trial will be whether the adjuvant treatment has any benefit for patients with lymph node metastases and its cost-effectiveness, but also the impact of this treatment on the patient's quality of life.

Quality of life

Although the new systemic therapies are promising, they often come with severe side effects.[46,50]

These therapies might prolong survival by a few months, but they often deteriorate the patient's quality of life. Besides side effects, melanoma patients experience numerous problems in physical, emotional, social, practical, and spiritual functioning.[53,54] Quality of life has become increasingly important in clinical research and it is expected to become even more important in the future. Future trials analyzing any surgical or medical therapies in melanoma patients will, besides the focus on survival and cost-effectiveness, focus on the quality of life of the patient.

Table 2. An example of a synoptic structured pathology report for a primary cutaneous melanoma

Pathological feature	**Example**
Sex	Female
Site	Right leg
Diagnosis	Melanoma
Histological subtype	Superficial spreading melanoma
Vertical growth phase	Present
Breslow thickness	2.6mm
Ulceration (diameter in mm)	Present (3.3mm)
Dermal mitotic index (per mm^2)	4 per mm^2
Clark level	IV
Vascular or lymphatic invasion	Absent
Neurotropism	Present
Desmoplasia (% of dermal invasive tumor)	Absent
Satellites	Absent
Features or regression	
Early (TILs)	Mild and focal (non-brisk)
Intermediate (angiofibroplasia ± TILs)	Absent
Late (fibrosis and loss of rete ridges)	Absent
Predominant cell type	Epithelioid
Associated nevus	Dysplastic compound nevus
Nearest lateral margin to *insitu* component	1.4mm
Nearest lateral margin to dermal invasive component	3.2mm
Distance from tumor to deep margin	5.3mm
Solar elastosis	Mild (1+)

TIL: tumor-infiltrating lymphocyte

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Appendices

List of publications

Dankwoord

Curriculum vitae

List of publications

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Curriculum vitae

Maarten Niebling werd geboren op 20 februari 1986 te Maastricht. Hij groeide op in Epe als zoon van Marianne en Rob Niebling en als broer van Carolien en Noortje. Na het behalen van zijn vwo-diploma aan de St Bernardus school te Epe in 2004, studeerde hij Geneeskunde aan de Rijksuniversiteit van Groningen (2004-2011).

Tijdens zijn studie werd al snel duidelijk dat hij een grote interesse had in de chirurgie. In zijn laatste studiejaar volgde hij daarom zijn semi-arts stage op de afdeling Chirurgie van het Deventer Ziekenhuis. Na zijn stage in Deventer heeft hij bij het Melanoma Institute of Australia te Sydney, onder begeleiding van Prof. dr. J.F. Thompson, een jaar onderzoek gedaan naar de diagnostiek en behandeling van het melanoom, en daarmee een grondige basis gelegd voor zijn promotie op dit onderwerp. Vervolgens is hij verder gegaan met zijn promotieonderzoek op de afdeling Oncologische Chirurgie van het UMCG te Groningen, onder begeleiding van Prof. dr. H.J. Hoekstra.

Op dit moment is hij werkzaam in het Sint Lucas Andreas Ziekenhuis te Amsterdam als ANIOS op de afdeling Chirurgie. Vanaf september 2014 hoopt hij samen met zijn vriendin Jorien een nieuw avontuur aan te gaan en als ANIOS Chirurgie te gaan werken in Zuid-Afrika.